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ENVIRONMENTAL FATE AND BIOLOGICAL CONSEQUENCES OF CHEMICALS RELATED TO AIR FORCE ACTIVITIES

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The design and evaluation of terrestrial ecosystems containing intact soil cores was shown to be an effective approach for predicting the fate and effects of hydrocarbon materials in simulated land spills. The 100-centimeter by 10-centimeter test systems provide sampling capabilities at 10-centimeter depth intervals in soil cores. Laterally inserted probes permit positioning of sampling boats containing hydrocarbon absorption materials to sample soil core leachates. Using a selective resin in the probes and gas chromatography techniques, the migration and effects of shale-derived and model jet fuels were monitored throughout soil columns. Following numerous recoverability studies, XAD-7 resin was selected for tracking these hydrocarbons.

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Hydrocarbons were monitored in the soil cores from 10 centimeters to 60 centimeters. The majority of components selectively monitored in the shale-derived JP-4 jet fuel study were transported to a depth of 10 centimeters. In small concentrations, two shale-derived JP-4 components were present at a 50-centimeter soil depth on day 252. The majority of model JP-5 jet fuel components were also transported to a depth of 10 centimeters. On day 173 of the experiment, three components of model JP-5 were still present at a depth of 30 centimeters. Past that day no components were detected. This study simulated a spill of 100 milliliters of a 10% solution over a 78.5-square centimeter surface. Sample treated cores were tested under laboratory conditions and in field studies and using natural weather conditions for comparative tests. No significant differences were noted between field and laboratory conditions. Biological consequences of jet fuels were interpreted in the model ecosystems by carbon dioxide analyses, toxicity, and mutagenicity testing. Both jet fuels cause an increase in carbon dioxide output from the headspace over the ecosystems when compared to control columns. Model JP-5 is very toxic to microarthropods. Both jet fuels, however, show low toxicity to cultured mammalian cells in a clonal assay. Jet fuel JP-4 was found to be mutagenic to one strain of *Salmonella typhimurium* in the Ames test. The soil core ecosystems proved to be very functional for evaluating simulated hydrocarbon spills.

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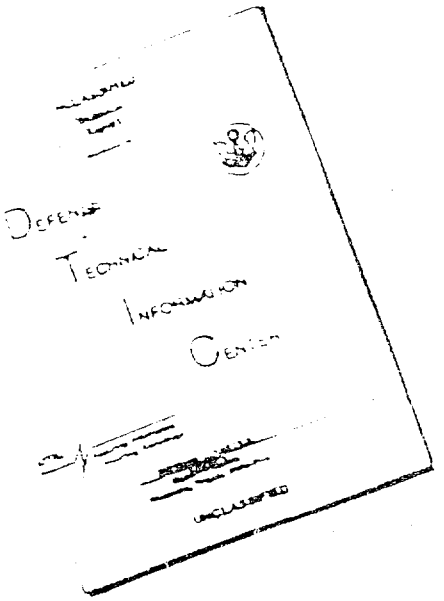

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EXECUTIVE SUMMARY

Terrestrial ecosystems containing intact soil cores have shown effective utility for evaluating vertical migration of hydrocarbon chemicals in soil. Sampling probes containing selective adsorbants have demonstrated that the hydrocarbon components of jet fuels can be recovered for analysis. Gas chromatographic techniques are very sensitive for tracing and quantifying hydrocarbon movement in the soil cores. Soil microarthropods are very sensitive organisms for testing the toxicity of jet fuel components. Carbon dioxide evolution into the headspace above the jet fuel treated soil cores increases and may be an indicator of stress to soil flora and fauna. The paths and rates of transport of aqueous leachates through soil cores change dramatically with time because of fauna borings and tunneling as well as by other physical causes. The hydrocarbon components of jet fuels migrate to varying depths and quantities independent of one another and appear to be independent of aqueous leachate movement. Microbial degradation of Model JP-5 jet fuel appears to have occurred in culture flasks containing jet fuels inoculated with cultures of soil organisms.

TEST SYSTEM DEVELOPED

- Model terrestrial ecosystems were designed and evaluated.
- Intact soil cores were obtained for tests.
- Sampling systems were designed using lateral probes at 10-cm vertical core depths.
- Hydrocarbon adsorbents were used in sampling boats for recovery of hydrocarbon components.

TESTS PERFORMED

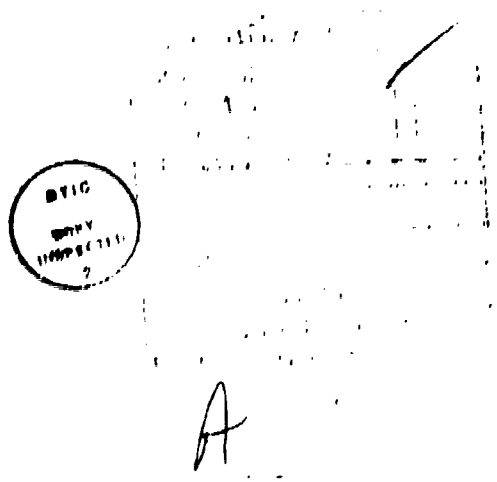
- Shale-derived jet fuel JP-4, Model JP-5 and hydrazine were evaluated for fate in terrestrial ecosystems.
- Jet fuel components were tested for migration and degradation in soil.
- Hydrocarbon components were sampled at different depths in soil cores.

- Gas chromatographic analyses of recovered hydrocarbons were performed.
- Microbial degradation studies were performed.
- Acute toxicity studies were performed on jet fuels.
- Microbial mutagenicity tests of jet fuels were performed.
- Microarthropods were used as indicators of jet fuel toxicity to biota in soil cores.
- Carbon dioxide evolution into core headspace was used as an indicator of stress to biota of ecosystem.
- Aqueous leachate quantities were measured at various depths to study transport mechanisms.
- Jet fuels were evaluated in outdoor and indoor soil core ecosystems.

RESULTS

- Both JP-4- and JP-5-dosed cores initially show a stressed condition as indicated by an increased rate in CO_2 production followed by a rate of CO_2 production comparable with the controls.
- JP-4 and JP-5 jet fuels have low acute cytotoxicity using cultured rodent and human cells.
- JP-4 is mutagenic to one Ames bacterial strain.
- JP-5 is very toxic to soil microarthropods at doses of 6.6 mL/ft^2 and 3.3 mL/ft^2. Possible alterations in growth processes could be occurring.
- Soil microbes are able to degrade JP-5 in cultures inoculated with soil organisms.
- Movement of JP-5 in the laboratory occurred to a depth of 50 cm with the majority of hydrocarbons being transported in the first 10 cm. Hydrocarbon components did not persist past the 131st day of the experiment.
- The outdoor soil core showed movement of JP-5 to a 30-cm depth. The majority of the fuel hydrocarbons were seen at 10, 20, and 30 cm. Up to the 173rd day of the experiment hydrocarbons were detectable in the core.

- The core dosed with JP-4 showed hydrocarbon movement down to 50 cm. Only a few hydrocarbons were present at this depth.
- Water leachate quantities and depths of transport varied considerably among all cores tested.
- Water leachate volumes recovered appear to be independent of hydrocarbon leachate quantities.
- Hydrazine was not found to migrate into soil cores.



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1. INTRODUCTION

1.1 BACKGROUND

Many man-made chemicals or chemicals related to man's activity are potential hazards to human health and to the welfare of other biological organisms when released into natural terrestrial and aquatic ecosystems. To assess the impact of these compounds and future materials, cost-effective yet valid laboratory methods using model ecosystems have been investigated by many researchers (see the bibliography). No one ideal system has been identified; however, specific systems for specific compounds have been identified by the research efforts of these scientists.

This research program is designed to develop methodology to aid in the prediction of the fate and biological effects of released foreign materials on soil surfaces and to assess their fate and migration through the soil and into the groundwater. The intent of this research is to develop a valid approach using a laboratory system to assess the fate and effects of test chemicals before they are widely used in the environment.

In order to assess the effects of these chemicals in the environment, factors which could impair the ecological welfare of living organisms were identified. The quantity of material and its degree of toxicity, mutagenicity, biodegradability, mobility, persistence, and bioaccumulation are such factors considered significant in the assessment of potential damage to the environment. Our model ecosystem is designed to accommodate the monitoring of such factors.

The first year of investigation was devoted primarily to development of both a laboratory system and a protocol for performing actual environmental fate experiments of specific materials [1]. Some initial experiments were performed to test the system and to develop test procedures rather than to acquire final test data.

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[1] Ross, W. D., Hillan, W. J., Wininger, M. T., McMillin, C. R., Gridley, J. A., Kebe, S. C., Aubuchon, J. J., Spillman, J. E., Gohmann, C. M., and Hughes, G. A. Environmental fate and biological consequences of chemicals related to Air Force activities. Dayton, Ohio; Monsanto Research Corporation; September 1980. 33 p. Contract F49620-79-C-0207, Report No. MRC-DA-1000.

In the second year of study [2], modification of equipment, refinement of experimental procedures, and evaluation of the soil ecosystems using JP-4 jet engine fuels were performed. Further analytical data involving a model JP-5 and hydrazine were generated in the final year of study. All areas of study are covered in detail in this report.

1.2 OBJECTIVES

The specific objectives of this research program were to:

- Develop a valid protocol for assessing the environmental effects of chemicals related to Air Force activities in a laboratory terrestrial ecosystem to provide information for preparation of effective environmental impact statements.
- Develop and evaluate a laboratory model terrestrial ecosystem that can be used to determine the effects of these materials on soil biota.
- Develop *in situ* sampling systems that will permit the efficient recovery of hydrocarbon components and/or their degradation products as a test material progresses through an intact soil core in the ecosystem.
- Assess the bioactivity of these products and metabolites for health and ecological effects as the test material moves through the model ecosystem.
- Chemically characterize the bioactive components by instrumental analytical methods.
- Finally, validate the test system by field studies.

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[2] Ross, W. D., Hillan, W. J., Wininger, M. T., Gridley, J. A., and Fullenkamp, J. M. Environmental fate and biological consequences of chemicals related to Air Force activities. Dayton, Ohio; Monsanto Research Corporation; September 1981. 50 p. Contract F49620-79-C-0207, Report No. MRC-DA-1092.

2. EXPERIMENTAL

2.1 DESIGN AND FABRICATION OF MODEL ECOSYSTEM

Many investigations have been performed by other researchers using variations on laboratory terrestrial ecosystems (see bibliography). Many of the test systems and procedures use standardized soils, biota, and meteorological conditions. Because of the complexity and variety of different soil and water ecosystems in nature, we have chosen to investigate the problem using a more empirical approach where the actual site of potential spills is simulated in the laboratory. This was done by acquiring soil cores and meteorological history from the site of concern. These experimental conditions were used to mimic the field conditions as closely as possible.

A prototype laboratory-model terrestrial ecosystem was designed and fabricated as shown in Figure 1. Several ecosystems are shown set up in the laboratory in Figure 2. The material of construction was polyvinyl chloride,[a] which proved to have the excellent physical strength properties required for filling with intact soil cores. The material is also transparent, permitting visual observation of the *in situ* soil core. Models are shown set up in the laboratory in Figure 2.

Concern over the potential bioactivity of the polyvinyl chloride, polymeric material led to an evaluation of small fragments (25 µg) of the material for mutagenicity using the Ames microbial test. Acute cytotoxicity was also evaluated using the CHO clonal assay. No bioactivity was seen in either bioassay.

The original ecosystem models are 1 m high and 10 cm in diameter with a wall thickness of 0.5 cm. Removable top and bottom caps are fabricated from polyvinyl chloride. The top has a 1/4-inch Swagelok fitting for positioning the 100-mL water/sample reservoir. It contains a stainless steel water-distributing system for simulating rainfall on the surface of the soil core. Figure 3 is a photograph of the water distribution system inside the top cap. The bottom also contains a 1/4-inch Swagelok fitting for the exit of aqueous leachate.

The body of the system contains seven, 2-cm-diameter sampling ports at 10-cm vertical intervals, oriented 60° apart. Male Swagelok fittings made of 316 stainless steel, are threaded into the lower six ports to accommodate 1.2-cm-diameter stainless steel probes. The probes are hollow tubes 18.5 cm long with pointed tips that allow insertion into the soil core (see Figure 4). A hole, 1 cm in diameter, is located 1 cm from the tip of each probe

[a] Excellon-R-4000, purchased from Dayton Plastics, Dayton, Ohio.

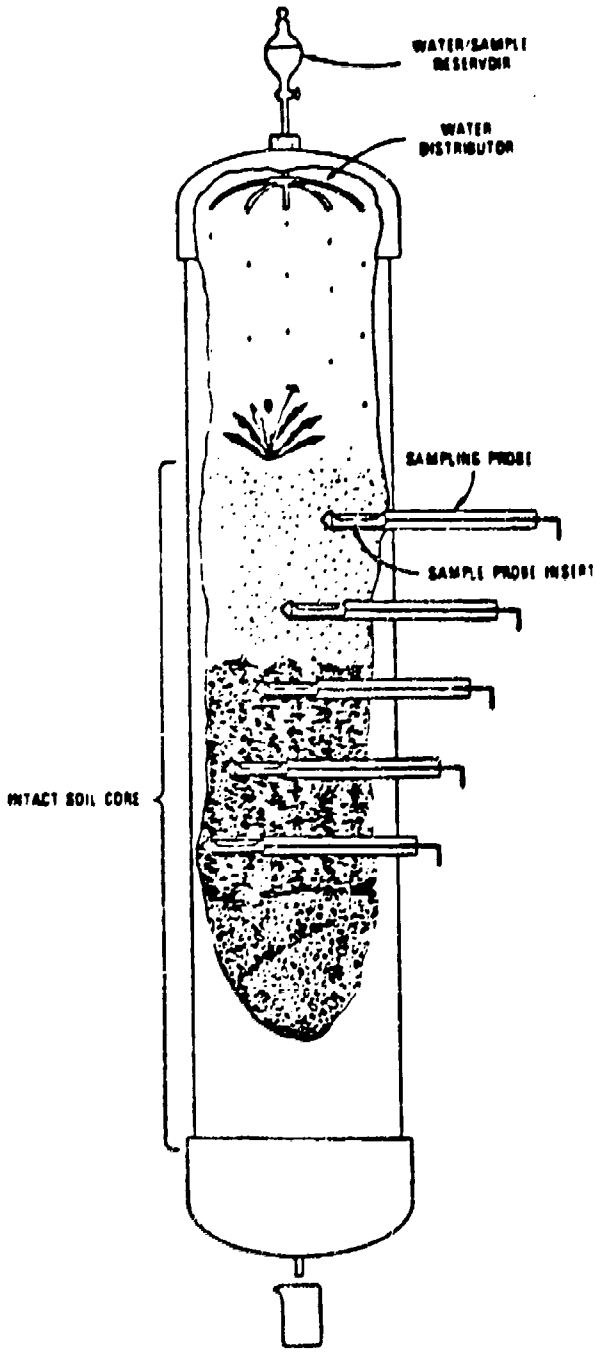


Figure 1. Laboratory terrestrial ecosystem design.

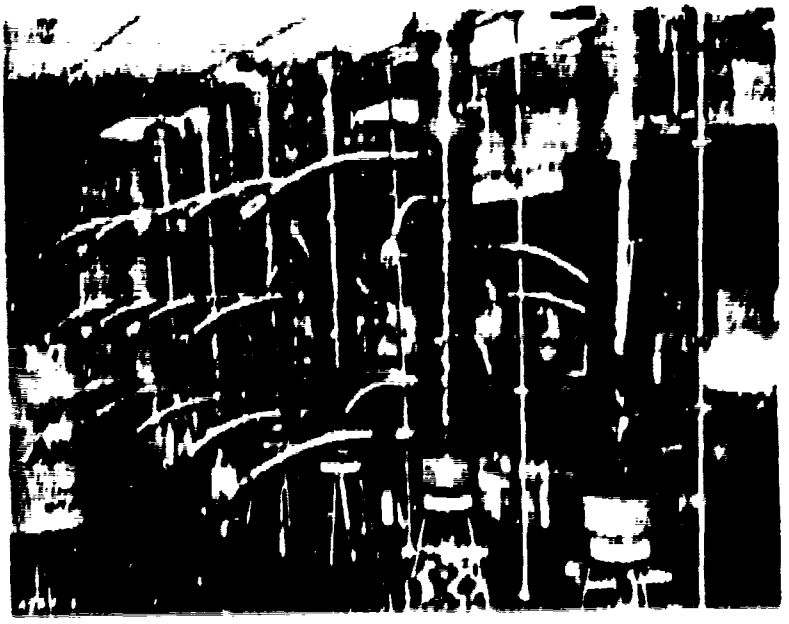


Figure 2. Laboratory ecosystem models.

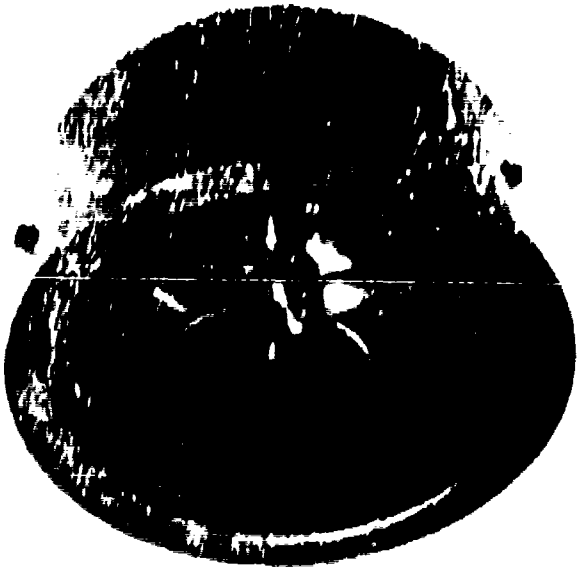


Figure 3. Water distribution system in top cap of ecosystem.

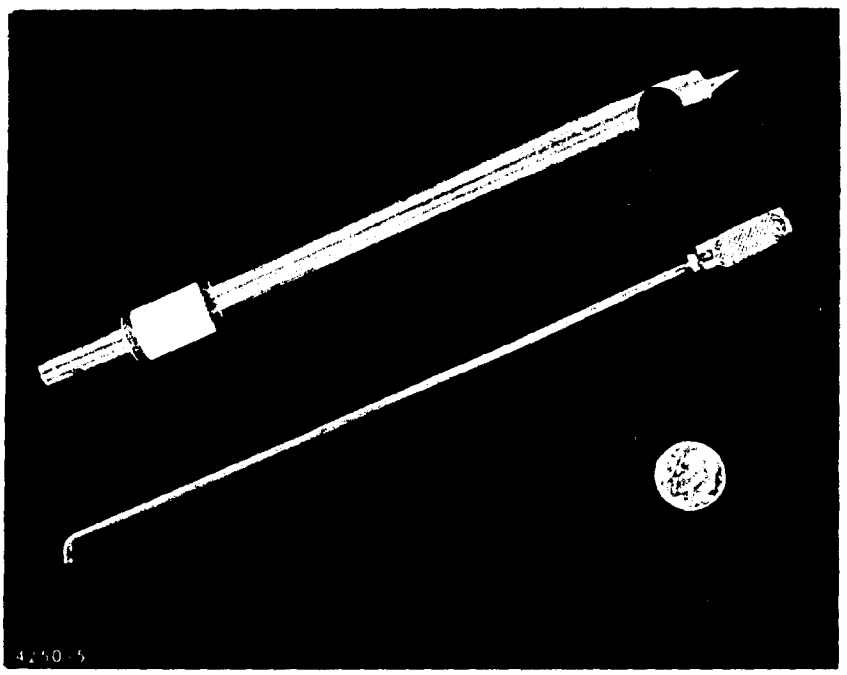


Figure 4. Sample probe assembly.

to allow passage of leachate into the probe and out the distal end. Teflon tubing fitted over the distal end allows collection of leachate in French square bottles.

The hollow probes accept sampling boat inserts designed to contain adsorbent materials. The sampling boats, machined from 1-cm-diameter stainless steel rods, have 1.8-cm by 0.7-cm ports cut through their diameters (see Figure 5). Stainless steel screens positioned on either side of each port hold 1.25 cm^3 of XAD resins or other adsorbents in place. The leachate flowing into the sampling probe can flow freely through the sampling material and out the probe tube. Each sampling boat is threaded onto the end of a stainless steel rod 3 mm in diameter and 23 cm long.

The top port in the system contains a 1/4-inch (0.6-cm) male, stainless steel Swagelok fitting and within it is a Teflon/silicone septum. A 1/4-inch (0.6-cm) female fitting is coupled firmly over this septum. This port enables a gas-tight syringe to be inserted through the septum and headspace gases removed for subsequent injection into the gas chromatograph. Further descriptions of headspace gas analysis by this method and an alternative method are presented in Section 2.4.5.

2.1.1 Modification of Initial Ecosystem Design

Several changes in the initial design of the system were deemed necessary after evaluating the ecosystem. First the sampling

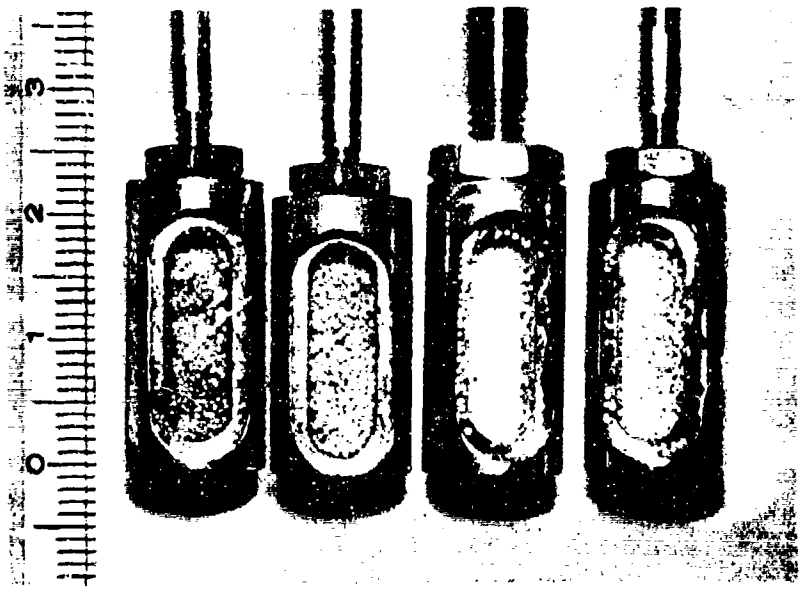


Figure 5. Sample probe sampling boat containing adsorbent inserts.

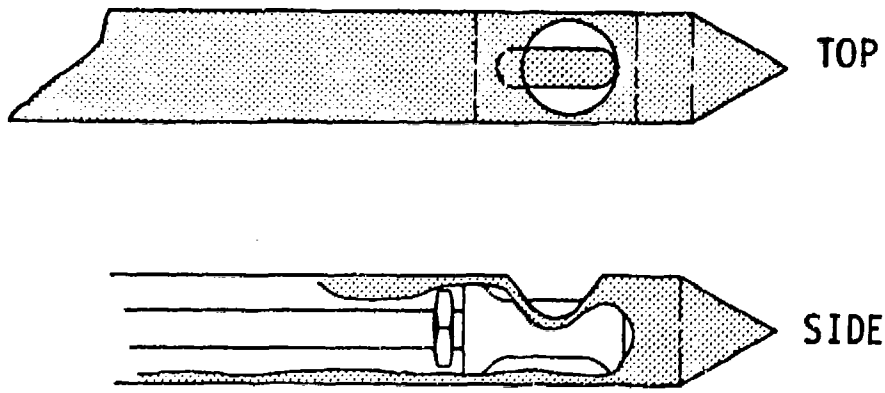
probes of the latest ecosystem design have been modified by placing the probes at a slight downward angle (10° below horizontal) to facilitate immediate flow of leachate to the sampling bottles. Prior to this change, leachate would occasionally fill up the probes because of surface tension before running out, causing misreading of leachate migration rate data.

The sampling probe and inserts were also made, as shown in Figure 6. This modification (Figure 6B) addressed a need for the option of permitting aqueous leachate to pass through the adsorbent for recovery of organic compounds without impeding the flow of water to lower soil depths. The initially designed probe (Figure 6A) permits shunting of the leachate laterally to the outside for monitoring flow rates and volumes. Depending on the particular study required, either option is now available.

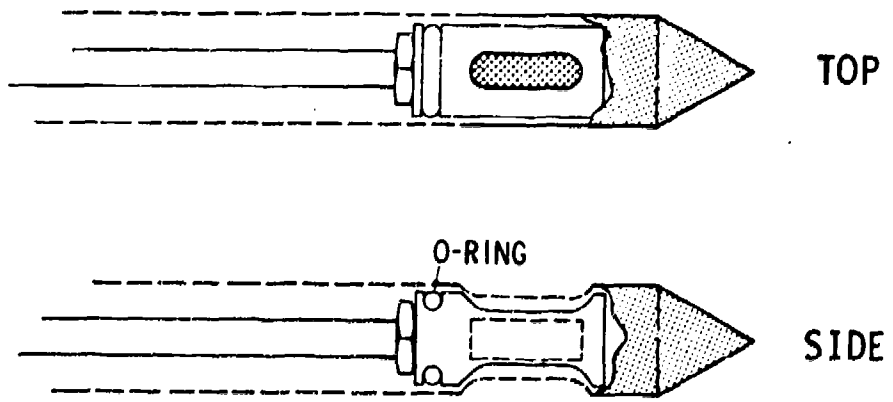
In preparation for monitoring headspace vapors above the soil cores, ecosystems were shortened to reduce headspace. The original 100-cm tube was cut to 75 cm. This modified ecosystem aids in the concentration of volatile components into a smaller headspace volume. Volatile degradation products or volatile components of the test material are then recovered and analyzed.

2.1.2 Soil Core Placement in Ecosystem

In preparation for filling the laboratory ecosystem models (the polyvinyl chloride tubes) with intact soil cores, trenches 4 ft



A. INITIAL DESIGN



B. MODIFIED DESIGN

Figure 6. Illustration of sampling probe. A - original and B - modified design.

by 1 ft by 3 ft deep (121.9 cm by 30.5 cm by 91.4 cm) were dug in the soil adjacent to the site where the cores were to be taken. The uncapped tubes were placed on end about 1 ft from the edge of the hole, and a 2-ft-long (61-cm) 2 in. by 4 in. (5.1 cm by 10.2 cm) soft pine board was placed across their tops. A sledge hammer was used to drive the tubes into the soil to a depth of 73 cm. The tops were then pushed laterally back and forth, freeing the tubes containing the intact cores. Later, cores were obtained by driving the tubes into the ground at desired depths. Then holes were dug with a post hole digger adjacent to the filled tube. The bottom caps were put in place and the ecosystem models were taken into the laboratory.

2.2 DEVELOPMENT OF TESTING PROCEDURE FOR EVALUATING THE FATE OF MATERIALS

2.2.1 Evaluation of Prototype Model Ecosystem

Test procedures were investigated for determining the optimal method for evaluating the chemical transformation of test materials. Included are the extent and rate of transport through the soil core using simulated rainfall.

Initial studies involved the gravitational transport of 100 mL of water added to the top of the soil core by the water distribution system. Dry filter paper was placed in the probes and the time was recorded as each probe filter paper was wetted and as the water leachate began to run from the bottom of the ecosystem. The total time was about 4 hours. This indicated that little or no channeling was occurring at the soil/tube wall interfaces.

Additional preliminary work included use of a 100-mL aliquot of a solution of crystal violet (used as a visible tracer) to determine the sampler and adsorbent recovery efficiencies as well as the flow characteristics through the sampling probe and sampling insert. The sample was added by the sample distribution system. Samplers containing XAD-2 resin were inserted at 10, 20, 30, 40, and 50 cm below the soil surface. The 10- and 20-cm probes retained enough crystal violet to color the XAD-2. The lower probes and bottom leachate had no visible color.

Based on the results described above, the laboratory ecosystem was evaluated for (1) functional properties of the water distribution system, (2) water transport and permeability properties of the soil core, and (3) functional properties of sampling probes and adsorbents. Following the favorable outcome of the laboratory transport studies in our model ecosystem, efforts were then concentrated on the development of a leachate concentration method; a mechanism for studying stress and equilibration, and development of an analytical scheme.

2.2.2 Appropriate Method for Leachate Concentration

The potential toxic metabolites and degradation compounds are dilute in many of these studies, especially as the materials progress through the core. Therefore, it was essential to concentrate the materials for both bioactivity testing and chemical characterization. Three methods of concentration were investigated: adsorption, reverse osmosis (RO), and lyophilization. The XAD-adsorption methodology was determined to be the most useful method for collecting samples *in situ* and also for concentrating organic materials in the leachate. The other two techniques were evaluated for potential use in concentrating larger amounts of leachate as it passed through the ecosystem.

The adsorbent used in the adsorption study was Rohm and Haas Amberlite XAD-2, a low polarity styrene-divinylbenzene copolymer[a] which possesses the macroreticular characteristics necessary for high sorptive capacity. Its recovery efficiencies have been determined for over 80 organic compounds in water by Junk, et al. [3].

In this study, submutagenic amounts of acridine orange (170 ppb) were added to wastewater and passed through XAD-2 resin.

Figure 7 shows the apparatus used. The XAD was then desorbed with 5 mL of dimethylsulfoxide (DMSO), and the recovered material was applied to an Ames mutagenicity test. The concentration factor was calculated to be 198 times and the material gave a positive response in the Ames test.

Further experiments were performed to find the most efficient adsorbent for use in the sampling probes. Rohm and Haas macroreticular XAD resins are well recognized as very good adsorbents for organic materials in aqueous systems. Some XAD materials are more efficient in the recovery of specific organic chemical classes than others, and we evaluated XAD-2, -4, -7, and -8 seeking the best adsorbent for the recovery of shale-derived JP-4. The XAD-7 adsorbent was found to be most efficient in recovering JP-4 from aqueous solutions, and was selected to be used throughout the jet fuel investigation.

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[a] Amberlite XAD Macroreticular Adsorbents, Rohm and Haas Research Division, Philadelphia, Pennsylvania.

[3] Junk, G. A., Richard, J. J., Grieser, M. D., Witiak, D., Witiak, J. L., Arguello, M. D., Vick, R., Svec, H. J., Firtz, J. S., and Calder, G. V. Use of macroreticular resins in the analysis of water for trace organic contaminants. Journal of Chromatography. 99:745-762, 1974.

Figure 7. XAD concentration evaluation apparatus.

Similar approaches were used to evaluate lyophilization and reverse osmosis techniques for concentrating larger amounts of leachate materials recovered from the soil core ecosystem. These two concentration techniques are usable but require much processing time and entail the use of more expensive equipment. Figures 8 and 9 are photographs of the test equipment used.

2.2.3 Determination of a Mechanism for Studying Stress and Equilibration

To aid in the assessment of the indirect effects of chemical stress on the biota and to determine the adjustment of the biota to the laboratory conditions, the concentrations of calcium in the leachate and carbon dioxide in the headspace were monitored. At the end of the 3-week equilibration time, the trend was linear and the cores were ready for treatment by the test material.

Calcium, dissolved in the leachate from the ecosystems, was measured by sequestering it with disodium dihydrogen ethylenediamine tetraacetic acid (EDTA) and titrating [4]. The titration end

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[4] Calcium-method 215.2 (titrimetric, EDTA). In: Methods for chemical analysis of water and waste. Cincinnati, Ohio; U.S. Environmental Protection Agency; March 1979. p. 215.2-1 through 215.2-3. EPA-600/4-79-020.

Figure 8. Lyophilization apparatus evauated for concentration of leachate.

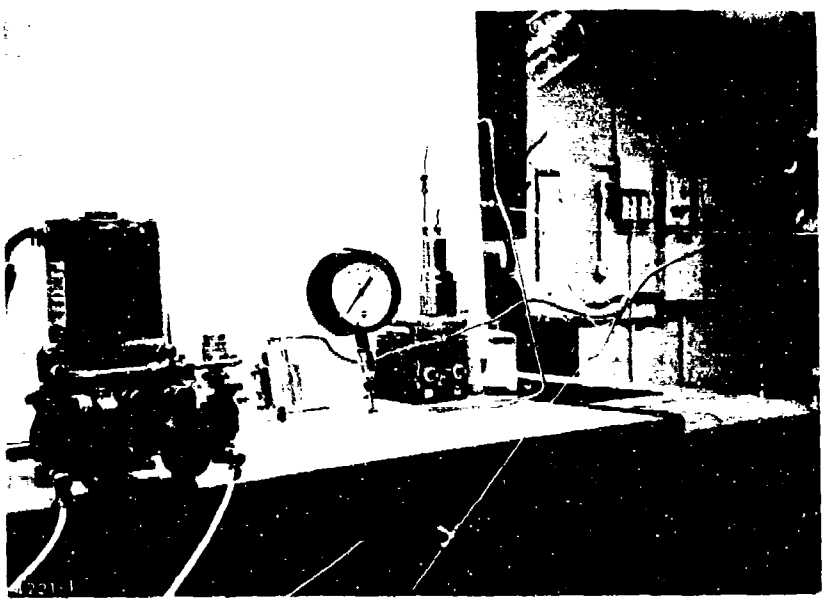

Figure 9. Reverse osmosis apparatus evaluated for concentration of leachate.

point is detected by means of an indicator, eriochrome blue black R, which combines with calcium alone. The titration is performed by adjusting the pH to 12 with 1 N sodium hydroxide. The indicator is added with continuous stirring until an end point is reached.

Carbon dioxide in the headspace gases was measured to assess the equilibration of cores; i.e., stabilization to laboratory conditions, prior to treatment with a test compound. Carbon dioxide evolution is an indicator of biota activity and equilibration is assumed when a constant evolution is indicated. Carbon dioxide was also measured after treatment of the cores with test materials to note changes in headspace content, which were used as an indicator of stress to the biota or changes in metabolic activity of the organisms in the soil. It was decided that the change in carbon dioxide would be used as a determination of equilibration and stress. This was based on the ease with which data could be obtained. Calcium analysis requires leachate to be collected, which is not always possible with this test system.

2.2.4 Groundwater Simulation

Variations in groundwater could possibly affect lateral and vertical transport of test material through the soil. Studies were undertaken to mimic groundwater variations in the laboratory test systems by maintaining a reservoir of water adjacent to the ecosystems. A tube, running from the bottom of the reservoir to the bottom part of the ecosystem, was used to supply subsurface water to the soil core. Groundwater levels were changed by raising and lowering the water reservoir, simulating groundwater fluctuations. This procedure was not incorporated into the testing protocol.

2.2.5 Analytical Scheme

The initial development of a test procedure in the first phase of this investigation is diagrammed in Figure 10. Shale-derived JP-4 jet fuel was used as the test material.

Figure 11 is a chromatogram of the shale-derived JP-4 jet fuel prior to subjection to the terrestrial ecosystem. The gas chromatographic (GC) instrument conditions were as follows:

Chromatograph: Hewlett-Packard Model 5880A
Detector: flame ionization
Detector gases: H_2, 60 mL/min; air, 300 µL/min
Detector temperature: 200°C
Column: 6 ft x 1/8 in., stainless steel packed with 3% OV-1 on 100-120 mesh
Column packing: Chromosorb B WAW DMCS
Carrier flow: 40 µL/min
Temperature program: -50°C to 200°C @ 8°C/min

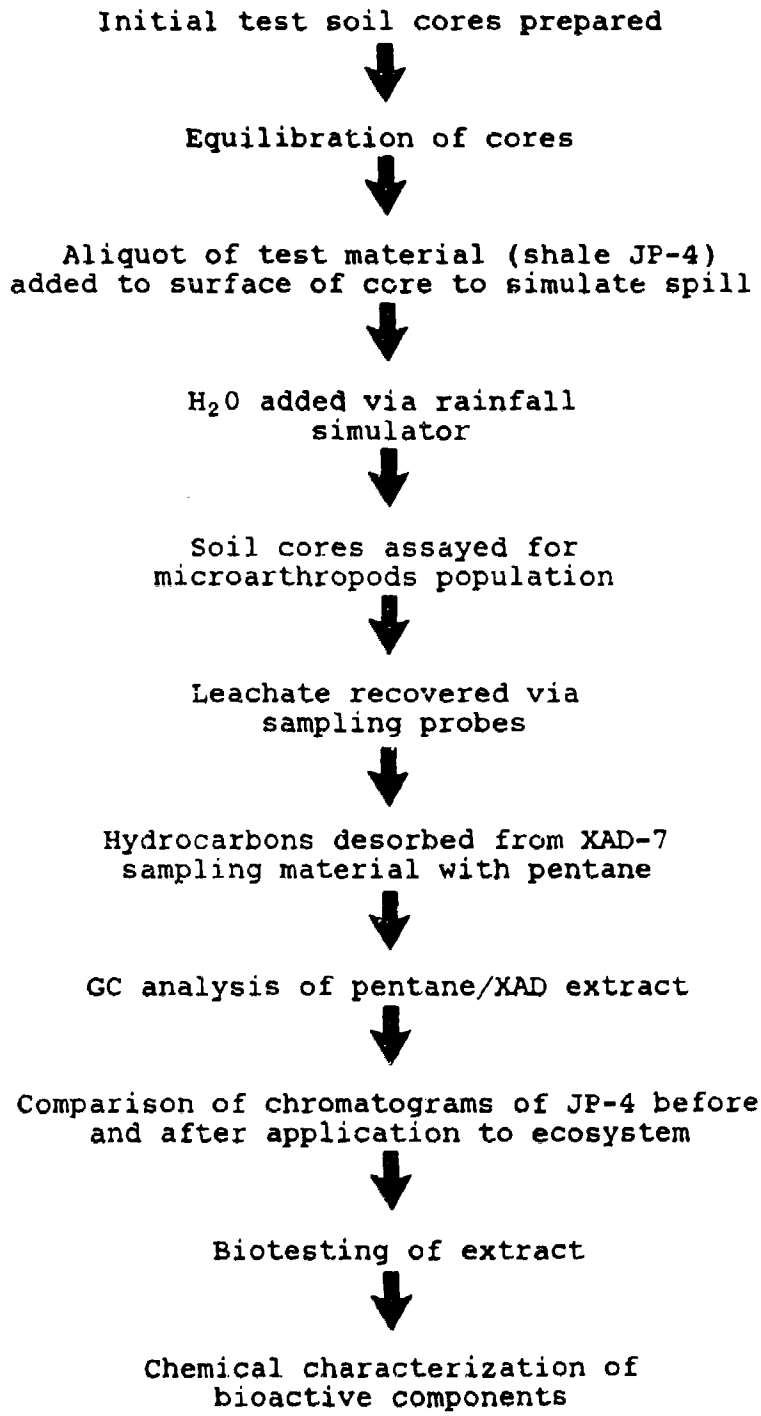

Figure 10. Suggested test procedure for evaluating shale-derived JP-4 in laboratory model terrestrial ecosystem.

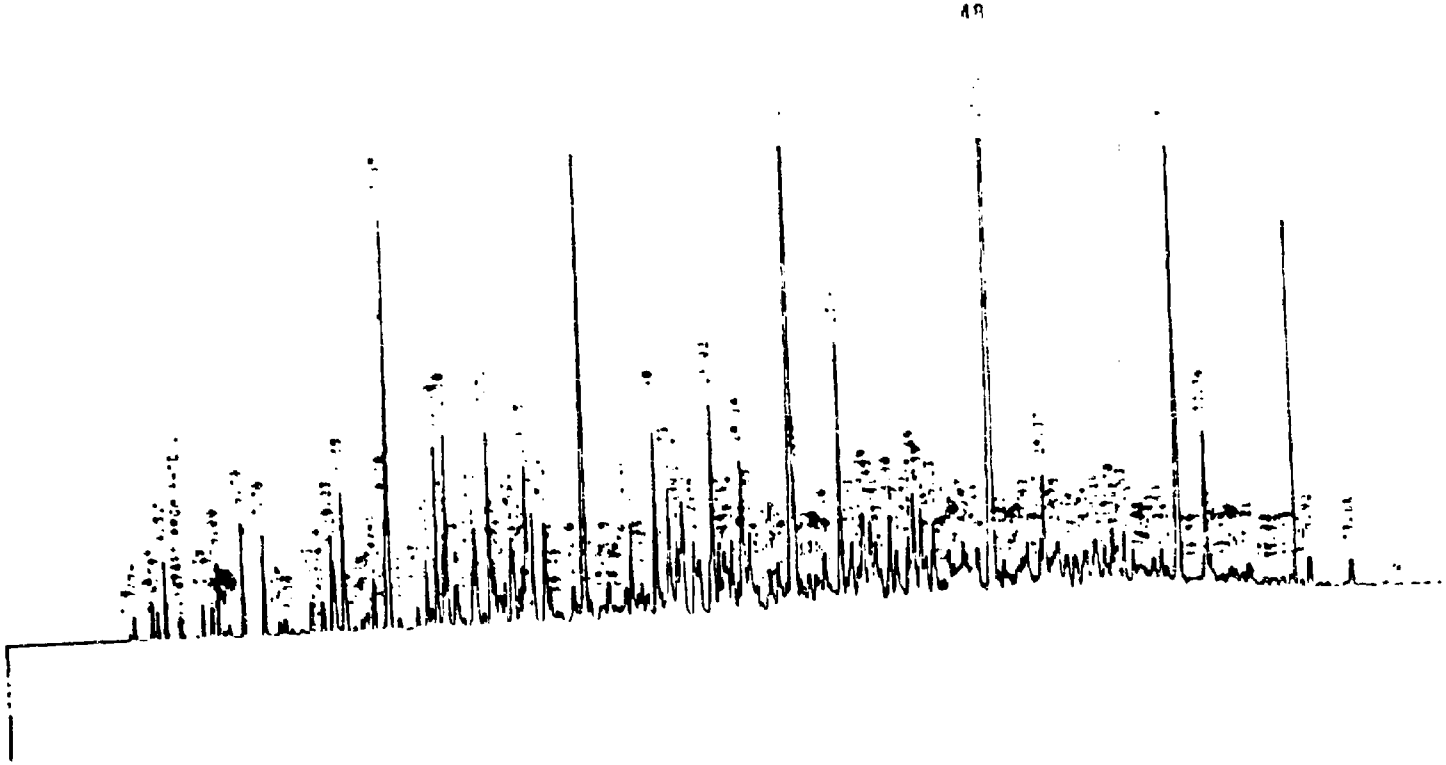

Figure 11. Gas chromatogram of shale-derived JP-4 jet fuel prior to subjection to ecosystem.

Table 1 presents the data recovered from this initial analysis of shale-derived JP-4 using the protocol described in Figure 10. Because of the complexity of this material, only the four major components were compared in this study. The amount of JP-4 added to the soil surface of the ecosystem was 5 mL (or 0.06 mL/cm^2). An aliquot of 100 mL of water as simulated rainfall was first added to the surface by the water distribution system. No aqueous leachate penetrated the core below the second sampling probe. The XAD-7 adsorbent contents of probes 1 and 2 were each desorbed with 2 mL of pentane 115 minutes after the addition of water to the core surface. Three microliters of each pentane solution were injected into the chromatograph and analyzed using the conditions described. These data in columns 2 and 3 of the table indicate that very small amounts of JP-4 components penetrated the top layer of soil of the ecosystem. Five days later another 100 mL of water was added to the surface of the core. After 3 hours the XAD-7 contents of probes 1 and 2 were desorbed with pentane and analyzed by gas chromatography. Again, the water penetrated the soil only to the depths of the first two probes. These data, recorded in columns 4 and 5, indicate that larger quantities of the JP-4 components were transported with the additional 100-mL aliquot of water.

TABLE 1. COMPARISON OF GAS CHROMATOGRAPHIC SEPARATED COMPONENTS OF JP-4 PRIOR TO TEST VERSUS RECOVERED MATERIAL FROM ECOSYSTEM SAMPLING PROBES

GC retention times of standard JP-4, minutes	Peak area from JP-4 XAD-7 recovery after H_2O addition			
	100 mL		200 mL	
	Probe 1	Probe 2	Probe 1	Probe 2
16.53	-	-	273	257
22.30	36	-	253	250
27.91	-	106	824	1,348
33.22	-	-	122	-

The GC peak areas of components measured, also indicated a selective recovery of components due either to selective adsorption or selective transport of the JP-4 components (see variation of components in probes 1 and 2, Table 1).

The above data were recovered from a preliminary experiment used to evaluate the prototype laboratory model ecosystem and to aid in the development of a test procedure. Additional experiments have been conducted and refined and are presented in Section 2.3, Bioanalytical Methodology, and Section 2.4, Analytical Methodology.

2.3 BIOANALYTICAL METHODOLOGY

Central to this investigation was the determination of the biological consequences; i.e., toxicity to living organisms of the starting test material, the chemical degradation products, and/or the biological metabolites formed as the test material progresses through the ecosystem. The bioactivity assessment determines toxicity to biota in the soil by the test materials; i.e., ecological effects and the potential for health effects problems. The mammalian cell clonal assay and the Ames mutagenicity test were used to test for health effects.

2.3.1 Carbon Dioxide Analysis

2.3.1.1 Purpose of Carbon Dioxide Analysis

As stated previously, carbon dioxide in the headspace gases was measured to assess the equilibration of the cores; i.e., stabilization to laboratory conditions prior to treatment with a test compound. Carbon dioxide was also measured after treatment of the cores with test materials to note the effects of chemicals on CO_2 evolution in headspace gases. This method was used as an indicator of stress to the biota and to study changes in metabolic activity of organisms in the soil.

2.3.1.2 Determination of Carbon Dioxide Evolution

The carbon dioxide evolved from the metabolic activities of biota in the soil was measured three times per week. Alkali traps containing 5 mL of 0.8 N potassium hydroxide were suspended in the headspace of the ecosystem. The traps were removed and titrated with 0.3 N hydrochloric acid [5].

2.3.1.3 Effects of Dosing with Shale-Derived JP-4

Carbon dioxide evolution was monitored in each of four cores for over a half a year. Core 9 was a control core and was not dosed. Core 10 was dosed with 10 mL of shale-derived JP-4. Figure 12 is a plot of the accumulated carbon dioxide evolution for both cores. After dosing Core 10 on day 36, there was an increase in the rate of carbon dioxide evolution from that core (as indicated in Figure 12) which became evident on day 40. Table 2 contains the running accumulation of the carbon dioxide analysis data over time for Cores 9 and 10. These data indicate that after the increase in rate of carbon dioxide evolution by Core 10, this core, following

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[5] Titrimetric method for free carbon dioxide. In: Standard methods for the examination of water and wastewater, 14th ed., 1975. Washington, American Public Health Association, 1976, p. 298-300.

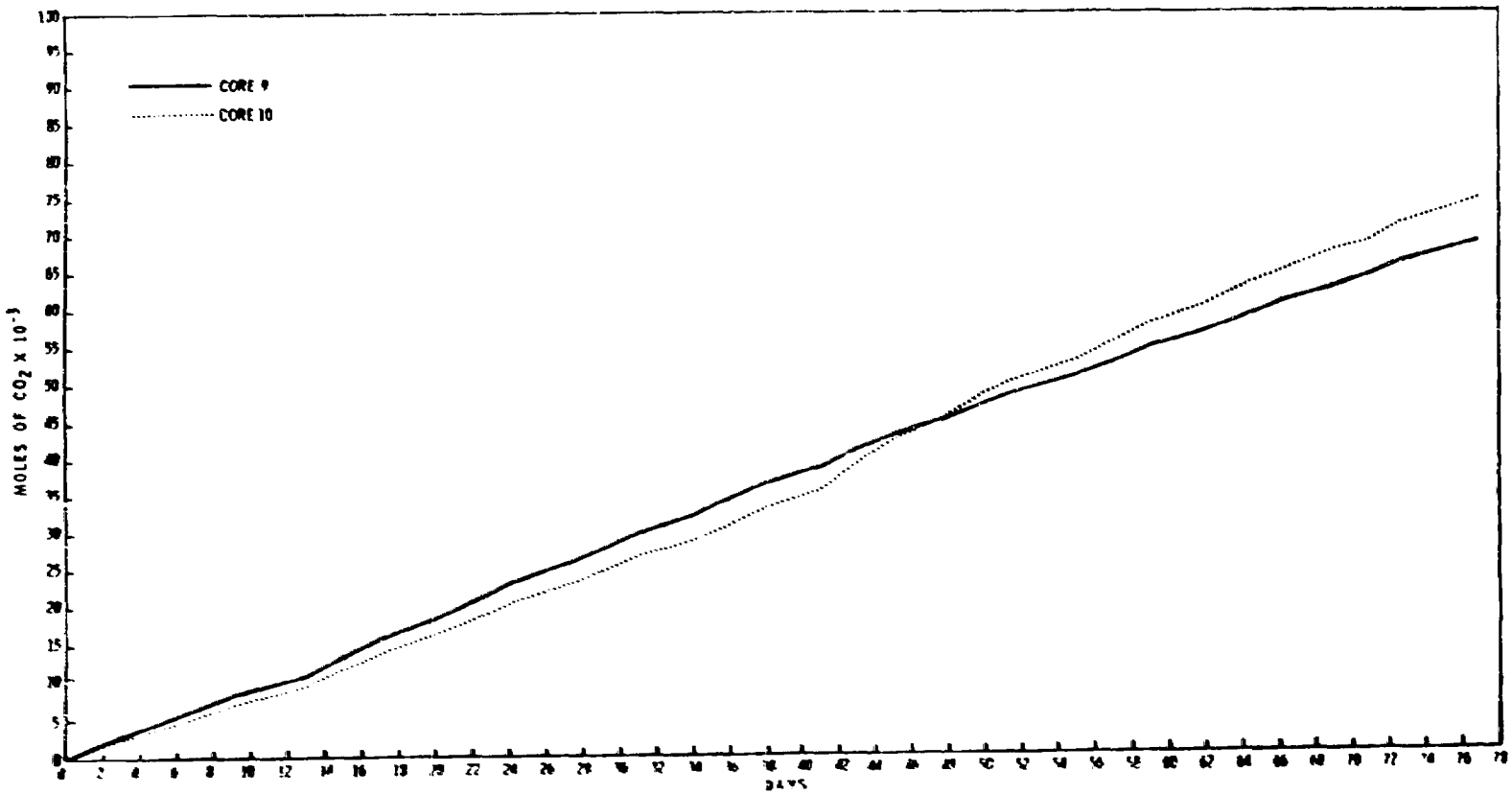

Figure 12. Comparison of carbon dioxide evolution in headspeace of soil cores 9 and 10.

TABLE 2. ACCUMULATIVE CO_2 ANALYSES OF CORES 9 AND 10 (moles of CO_2 x 10^{-3})

	Day																								
Core	3	6	9	13	15	17	20	22	24	27	29	31	34	36	38	41	43	45	48	50	52	55	57	59	62	64
9	3	5	8	11	14	16	19	21	23	26	28	30	32	35	37	39	41	43	45	47	49	51	53	55	57	59
10	2	5	7	10	12	14	16	18	21	23	25	27	29	31	33	36	39	42	45	48	51	53	55	58	60	63

	Day																								
Core	66	69	71	73	77	80	83	85	87	90	92	94	97	99	101	104	106	108	111	113	115	118	120	122	125	127
9	60	63	64	66	69	71	73	74	75	77	78	79	82	83	84	86	87	89	91	92	93	95	96	97	98	99
10	65	67	69	71	74	76	78	81	82	84	87	88	91	93	95	97	99	101	104	106	108	110	112	114	116	118

	Day																								
Core	129	132	134	136	139	141	143	146	148	150	153	155	160	162	164	167	169	171	174	176	178	181	184	187	189
9	101	102	103	104	106	107	108	110	111	112	114	115	118	120	121	123	124	125	127	128	129	130	132	133	134
10	120	123	125	126	128	130	132	135	137	138	141	143	146	149	150	152	154	156	158	160	161	164	166	167	168

	Day																								
Core	191	195	197	199	202	204	206	209	211	213	215	217	219	222	224	226	229	231	233	236	238	240	243	245	247
9	135	137	139	139	141	142	143	145	146	146	148	149	150	152	153	153	155	156	157	159	159	160	161	162	163
10	170	172	174	175	177	179	181	183	184	184	185	185	185	186	187	187	188	189	190	191	192	193	195	196	198

pH treatment, continues to produce CO_2 at a higher rate than the control core.

2.3.1.4 Effects of Dosing with Model JP-5

The other two cores which were monitored extensively for carbon dioxide evolution were Cores 11 and 12. Core 11 was the control core and Core 12 the dosed core. In this case, Core 12 was dosed with 10 mL of Model JP-5 on day 40. Figure 13 and Table 3 indicate a response similar to that of Core 10, the JP-4-treated column.

2.3.1.5 Results

Both the core dosed with shale-derived JP-4 and the core dosed with model JP-5 showed an increase in rate of carbon dioxide evolution within one day after application. Following the increase in CO_2 evolution rate, the carbon dioxide production of the dosed cores returned to a rate almost comparable to that of the control cores. Although carbon dioxide is a known byproduct of catabolism, this increase in production of carbon dioxide does not necessarily indicate biodegradation was taking place. It is possible the fuels were being utilized in the metabolic pathway and carbon dioxide was evolving as a result. However, the final stages of metabolism might be inhibited, in which case a decrease in the rate of the carbon dioxide formation would not occur immediately. Additionally, it is also possible the fuels were toxic to one species and not to another; for example, the arthropods and the bacteria, respectively. In such a case the bacteria would thrive as the arthopods decayed. Our soil ecosystem is a more complex testing tool than, for instance, a respirometer, with a number of possible factors contributing to the increase in carbon dioxide evolution. As such, we feel it offers a realistic approach to enviromental effects of compounds. Its drawback is that at best all that can be concluded is that the fuels are stressful to the ecosystems. Additional studies would be needed to determine if it is a positive or a negative stress to the environment. The following bioanalytical studies are supplementary to these data.

2.3.2 Acute Cytotoxicity Testing

2.3.2.1 Assessment of Optimum Method

The acute cytotoxicity of test material products was determined by the *in vitro* Chinese hamster ovary (CHO) cell clonal assay [6]

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[6] Wininger, M. T., Kulik, F. A., and Ross, W. D. *In vitro* clonal cytotoxicity assay for chemicals using Chinese hamster ovary cells (CHO-K1). Tissue Culture Association Manual. 5(2):1091-1093, 1979.

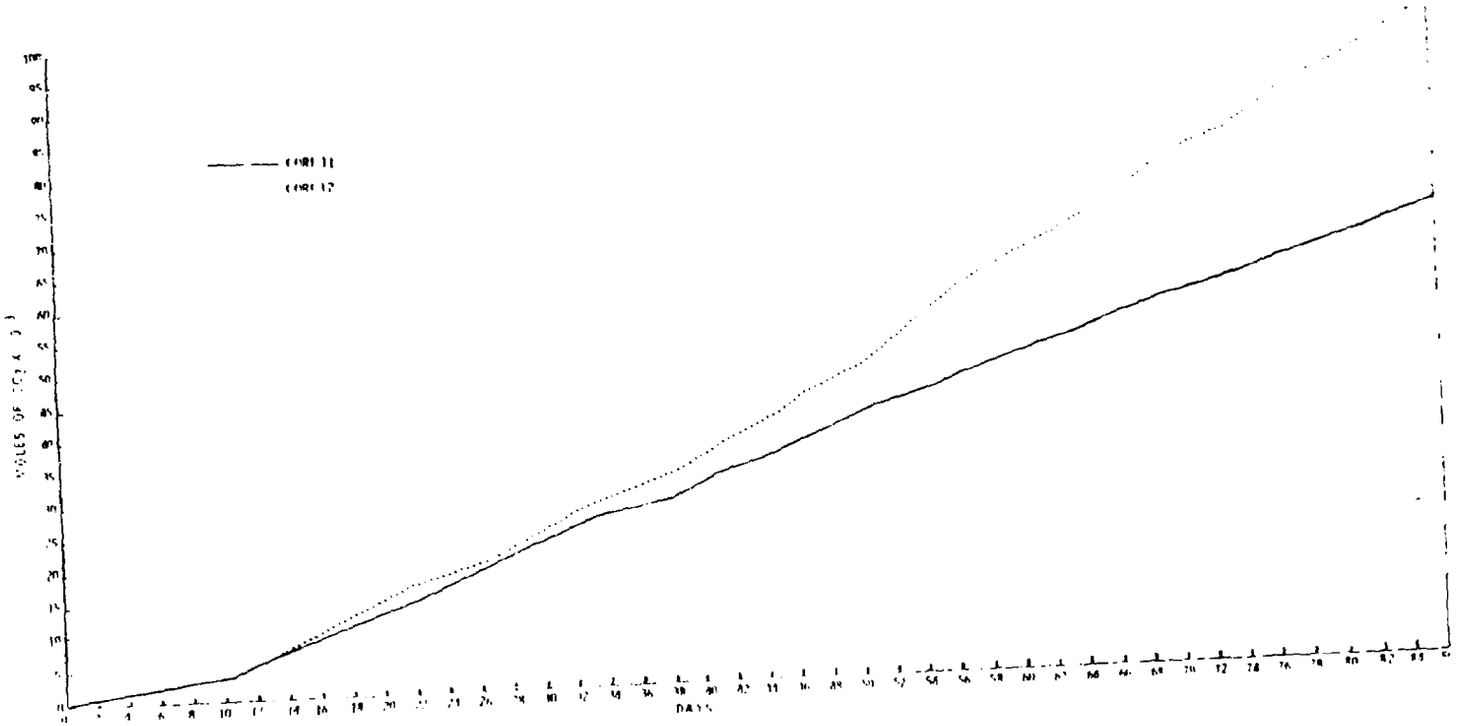

Figure 13. Comparison of carbon dioxide evolution in headspace of soil cores 11 and 12.

TABLE 3. ACCUMULATIVE CO_2 ANALYSES OF CORES 11 AND 12
(moles of CO_2 x 10^{-3})

	Day																				
Core	10	12	14	17	19	21	24	26	28	31	33	38	40	42	45	47	49	52	54	56	59	62
11	2	5	7	9	11	13	17	19	21	23	26	29	31	33	35	38	40	42	44	46	49	51
12	3	6	8	11	13	16	19	20	23	26	28	33	35	38	42	45	49	53	56	60	64	68

	Day																				
Core	65	67	69	73	75	77	80	82	84	87	89	91	94	96	98	101	103	105	108	110	112	115
11	54	56	58	60	62	64	66	68	69	71	73	75	77	79	80	82	84	86	88	89	91	93
12	72	76	79	83	86	90	93	96	99	102	105	108	111	114	117	120	123	126	129	132	135	138

	Day																				
Core	117	119	122	124	126	129	131	133	136	138	140	143	145	147	150	152	154	157	159	161	164
11	94	95	97	98	100	102	103	104	106	108	109	111	112	114	116	117	119	121	122	123	125
12	141	143	146	149	152	155	157		162	165	167	170	172	175	177	179	182	184	186	188	190

	Day																				
Core	166	168	171	173	175	178	180	182	185	187	189	192	194	196	199	201	203	206	208	209	212
11	127	128	129	130	132	133	134	135	137	138	139	141	142	143	145	146	147	149	150	151	152
12	192	194	195	198	200	202	204	206	208	209	212	214	216	218	220	221	224	226	228	230	232

and the mammalian cell agar overlay test [7]. The agar overlay test has been evaluated for the application of XAD adsorbent containing a known toxic material, acridine orange, and shale JP-4. Figure 14 illustrates the direct application of the XAD material. This test was used as a rapid spot test for toxic materials adsorbed on the XAD (located in the sampling probes) to the agar overlay test system.

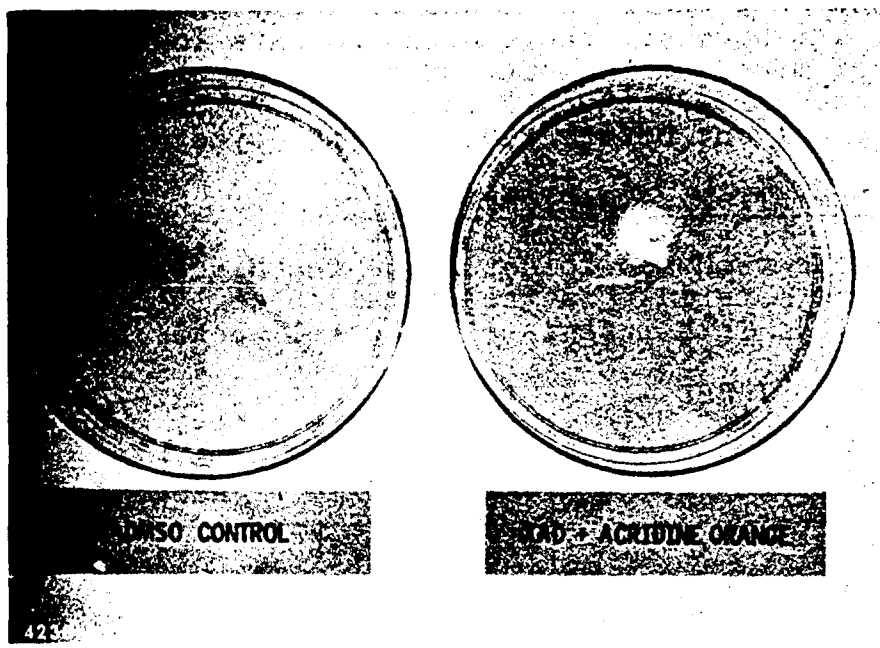

Figure 14. Agar overlay mammalian cell cultures; control culture and culture with XAD plus acridine orange.

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[7] Ross, W. D., Wininger, M. T., Hare, R. J., McMillin, C. R., and Gridley, J. A. A sensitive *in vitro* agar overlay cytotoxicity assay for elastomers using human epithelial cells. Presented at the 31st annual meeting of the Tissue Culture Association, June 1-5 1980, St. Louis, Missouri.

Initial studies to determine the optimum method for assessing acute cytotoxicity of test materials include the evaluation of shale-derived JP-4, petroleum-derived JP-4, and other standard organic compounds including chlorobenzene, phenyl ether, benzene, aniline, and ethylbenzene, representing various chemical classes. Due to its recovery ability, XAD-7 resin was used in the sampling probes. Therefore, this material was also used to evaluate the direct application of petroleum-derived JP-4 and XAD-7 to the acute toxicity test system. The procedure consisted of adding known amounts of the organic materials to known amounts of the resin.

In order to treat the mammalian cell test system with five increasing doses of the test material absorbed to the resin, various weights of the resin were used. The resin particulates were added directly to the CHO test and EC_{50} (effective concentration for 50% cell survival) was determined. Table 4 is a tabulation of the cytotoxicity test data for petroleum-derived JP-4 on XAD-7 and blank XAD-7 resin. Additionally, data on seven organic compounds added directly to the CHO clonal assay; i.e., not adsorbed to XAD-7, are also tabulated. This test demonstrated that acute cytotoxicity of absorbed JP-4 can be assessed directly on XAD-7 resin without desorbing it; however, these data indicate lower toxicity by nearly an order of magnitude. Thus, when incorporating the toxicity test with the ecosystem, it would be prudent to desorb the sample with an organic solvent before adding to the cytotoxicity test system.

2.3.2.2 Mammalian Cell Clonal Assay

The methodology chosen to determine the bioactivity of potentially toxic materials is the mammalian cell clonal assay technique [6]. This test is a rapid (<1 week) *in vitro* assay

TABLE 4. ACUTE CYTOTOXICITY DATA FOR STANDARD ORGANIC TEST MATERIAL

Compound	EC_{50}, µL/mL	Range tested, µL/mL
Blank XAD-7	No toxicity	2.49 - 12.45
JP-4 tank 15 on XAD[a] (petroleum-derived)	9	2.49 - 12.45
JP-4 tank 15 (neat) (petroleum-derived)	0.2	0.1 - 10
JP-4 (shale-derived)	0.2	0.1 - 10
Aniline	0.7	0.2 - 100
Benzene	1.0	0.2 - 100
Chlorobenzene	0.5	0.2 - 100
Phenyl ether	0.6	0.2 - 100
Ethylbenzene	0.2	0.2 - 100

[a]JP-4 tank 15 was adsorbed on XAD-7 resin which was added directly to the CHO agar overlay system.

which used mammalian cells, both hamster (CHO-K1) and human (D98S). The CHO cell line is nearly an ideal cell line because of its high cloning efficiency (95%) and excellent colony-forming properties; i.e., it forms tightly packed cells in discrete, easily distinguished colonies that can be counted automatically. The cell line exhibits continuous cell line growth properties, is highly sensitive to toxic materials (e.g., 50% survival of formed colonies [EC_{50}] at a concentration of 0.2 µg per mL of cadmium chloride) and shows toxicity correlation with *in vivo* test systems. The CHO clonal cytotoxicity test system has been validated by the analysis of many organic compounds, environmental samples, metal salts and known positive standard [8-11]. The CHO clonal method described here uses a colony counting end point. Results are rapidly quantified with an automated colony counter, and data are reduced via a computer program.

Cytotoxicity assays were performed on shale- and petroleum-derived JP-4, and model JP-5, using both CHO cells and D98S cells. This was a direct test of the compounds without sample absorption on XAD-7 or desorption with a solvent. The concentrations used are shown in Appendix A, Tables A-1 and A-2. Table A-1 shows processed acute cytoxicity data obtained from the computer for JP-4 (shale) using CHO-K1 cells; Table A-2 gives data from D98S cells. Figure A-1 in Appendix A is a graph comparing cytotoxicity curves of shale- and petroleum-derived JP-4 and model JP-5 as concentration of material added to the growth medium versus the percent colony formation. Both cell types are represented, CHO-K1 and D98S. The D98S cells were most sensitive to this sample. There was little difference between sensitivity of the CHO (hamster) cells versus the D98S (human) to the JP-4 whether derived from petroleum or shale. In all cases, the relative toxicity (effective

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[8] Wininger, M. T., Kulik, F. A., and Ross, W. D. *In vitro* clonal cytotoxicity assay using Chinese hamster ovary cells (CHO-K1) for testing environmental chemicals (abstract). *In Vitro*. 14:381, 1978.

[9] Wininger, M. T., Hare, R. J., Brautigam, G. F., Hill, J. T., Wilson, J. D., and Ross, W. D. Determination of acute cytotoxicity of elemental phosphorus (P_4) by *in vitro* clonal assay using Chinese hamster ovary cells (CHO-K1) (abstract) *In Vitro*. 15:199, 1979.

[10] Wininger, M. T., Kulik, F. A., and Ross, W. D. Short-term toxicity testing of chemicals using cultured animal cells. Ohio Journal of Science. 79:70, 1979.

[11] Campbell, J. A., Garrett, N. E., Huisingh, J. L., and Waters, M. D. Cellular toxicity of liquid effluents from textile mills. Presented at the Textile Industry Technology Symposium. Williamsburg, Virginia, December 1978.

concentration for 50% survival, EC_{50}) was in the low range by EPA toxicity criteria [12]. Table A-3 illustrates the relative cytotoxicities determined by clonal assay of the jet fuels tested can be readily compared to other toxic compounds.

2.3.2.3 Results

The relative cytotoxicities of shale-derived JP-4, petroleum-derived JP-4, and model JP-5 are all rated low by the mammalian cell clonal assay; i.e., JP-4 at 2.2×10^{-4} g/mL, model JP-5 at 1.4×10^{-4} g/mL.

2.3.3 Microbial Mutagenicity Testing

The potential mutagenicity of the test material products has been assessed with the Ames microbial test system [13].

2.3.3.1 Procedure

Testing of JP-4 in the Ames test involved a spot test, a toxicity test, and a plate incorporation test. A number of spot tests were run on a sample of JP-4. JP-4 was spotted onto a filter paper disk on the surface of agar. Tests were run with rat microsomal activation (S-9), with mouse activation, and without activation at 25 µL/plate in 4 strains of *Salmonella typhimurium*, TA98, TA100, TA1535, and TA1537. Based on colony counts the spot test showed no apparent mutagenicity but some toxicity.[a] A toxicity test was then performed to determine a range to test the sample in the plate incorporation test. The sample was diluted in ethanol to dosing concentrations of 10, 3, 1, 0.2, 0.04, and 0.01 µL/plate. Strain TA100 with and without rat microsomal activation was used for this test. The components were added to the top agar and poured over the surface of a minimal agar plate.

No toxicity was evident. As a result of the negative toxicity data, the plate incorporation test was run at the same concentration as the toxicity test. Four strains, TA98, TA100, TA1535, and

[a] It is not unusual to have a negative spot test and a positive plate incorporation test.

[12] IERL-RTP Procedures manual: Level 1 environmental assessment biological tests. Research Triangle Park, NC; U.S. Environmental Protection Agency; September 1980. p. 62. Contract No. 68-02-2681.

[13] Ames, B. N., McCann, J., and Yamasaki, E. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. Mutation Research. 31:347-364. 1975.

TA1537, with and without rat microsomal activation were run. All plates were counted.

2.3.3.2 Results

Significant (p = 0.01) results from the computer analysis (see Appendix A) include:

TA98 with S-9 having one value significantly greater than controls (p = 0.01) and a dose response significant (p = 0.01)

TA100 with S-9 having one value significantly greater than controls and a dose response not significant

TA1537 without S-9 having one value significantly greater than controls and a dose response significant

These three strains were retested at concentrations clustering around the significant value. An additional retest of TA1535 with S-9 at original concentrations was performed because solvent (ethanol) controls on the original test were outside the range acceptable in our laboratory. This retest showed no values significantly greater than control values.

The other retests showed:

TA98 with S-9: 3 values significantly greater than controls and a significant dose response. This meets our established criteria for a positive response.

TA100 with S-9: no values significantly greater than controls and a dose response not significant, considered to be a negative response.

TA1537 without S-9: toxicity occurred at 2 out of 3 doses tested, so not enough data were available for computer analysis. Normally, this would again be retested, had not one strain (TA98) already shown a positive response.

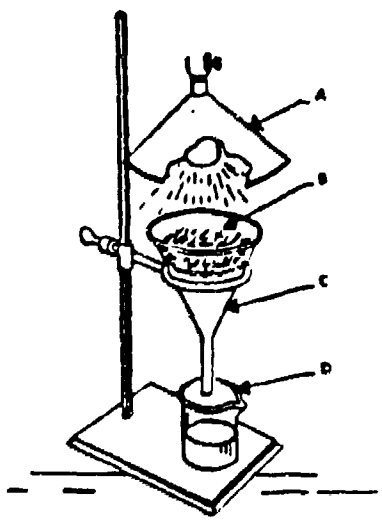
2.3.4 Microarthropod Toxicity Testing

Large numbers of small subsurface animals exist in soils, especially in the soil horizons that contain higher levels of natural organic material (i.e., the A and B soil horizons). The majority of all living animals in the soil are arthropods, and a high proportion of soil biota is made up of representatives of this phylum. The most important soil arthropods are termites, beetles, ants, flies, myriapods, springtails, and mites. These small animals can be used to indicate changes in the soil ecosystem brought about by

foreign chemicals. For this study, soil arthropods were selected for observation as indicators of the effects of chemicals on soil biota. Initial experiments also included soil nematodes [14].

2.3.4.1 Procedure

A relatively simple system used to isolate these soil microarthropods and obtain population counts is described by Pramer and Schmidt [14]. A lightbulb is used to dry the soil sample. This causes the living arthropods to migrate downward with the moisture until they drop through a screen supporting the soil sample and into a collecting reservoir. (See Figure 15).



BERLESE FUNNEL FOR EXTRACTING ARTHROPODS FROM SOIL AND LITTER: A, lamp, B, screen supporting litter, C, funnel, and D, collecting vessel containing alcohol.

Figure 15. Berlese funnel for extracting arthropods from soil and litter.

Cores used for the microarthropod toxicity testing were obtained in two fashions. The first ten cores (identified as a-j in Table 5) were obtained with a bulb digger. The weight of each core was taken immediately after removal from the soil and is indicated as "wet weight" in Table 5. These cores were not dosed with any compound and were used for the development of a population history. The second batch of ten cores (identified as k-t

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[14] Pramer, D., and Schmidt, E. L. Exercise 6 - arthropods. In: Experimental soil microbiology. Minneapolis, Burgess Publishing Company, 1964, 18-19.

TABLE 5. COMPARISON OF TOTAL NUMBER OF LIVING MICROARTHROPODS IN CORES DOSED WITH MODEL JP-5 AND CONTROL CORES

Undosed cores			Dosed cores			Undosed cores			Dosed cores		
ID	Wet weight, g	Total counts	ID	Dry weight, g	Total counts	ID	Dry weight, g	Total counts	ID	Dry weight, g	Total counts
a	271	87	k	214	1[b]	u	255	71	z	311	6[a]
b	282	56	l	-	187[b]	v	270	124	aa	250	6[a]
c	286	56	m	186	0	w	308	87	bb	297	6[a]
d	241	50[c]	n	197	24[b]	x	252	133	cc	248	13[a]
e	245	5[c]	o	186	1	y	198	107	dd	218	1
f	236	36	p	202	0						
g	270	53	q	240	0						
h	284	34	r	211[d]	0[d]						
i	251	61	s	-[d]	-[d]						
j	217	23	t	188	1[b]						

[a]Several microarthropods' exoskeletons appeared soft.

[b]All microarthropods' exoskeletons appeared soft as would appear after ecdysis.

[c]Integrity of the core was destroyed in transfer to the extraction apparatus.

[d]Could not remove core.

Note. Dashes indicate data not available.

in Table 5) were obtained with a small polyvinyl chloride tube, 7.5 cm in diameter. The soil was removed intact by first tapping the tube into the ground with a sledge hammer and then pulling the core upward while moving it from side to side. The cores were dosed in the laboratory with 2.4 mL of model JP-5 and extracted by the method described previously. Population counts were made on the 14th day after dosing. The weight of the cores, in these and all others to be mentioned, was taken after the extraction and is termed "dry weight." The change from wet weight to dry weight measurements was made in order to better quantify counts/soil weight and eliminate a possible step in which population count errors could occur. The third group of ten cores (identified as v-dd in Table 5) were obtained by the coring method described above. Half of the cores were dosed with 1.2 mL of model JP-5, and half were not dosed. These cores were sacrificed at various intervals. Cores u, v, z, and aa were sacrificed a week after dosing. During the second week after dosing, Cores w, x, bb, and cc were extracted. The final extraction occurred three weeks after dosing; and Cores y and dd were used at that time.

2.3.4.2 Results

There is a significant difference between the population counts of the dosed and undosed cores in all cases. The majority of the dosed cores show toxicity as indicated by the low population counts. In the few exceptions where the population counts were high for the dosed cores appearance of the microarthropod was altered. The change noticed was that the exoskeleton, normally a hard chitinous

material, was soft in these cases. The appearance of the soft exoskeleton resembles that of an arthropod after ecdysis (molting). These soil samples were obtained in the late fall. This is not a normal time for arthropods to molt. The most critical time for an insect's survival is during the molting process. An unscheduled molting for instance, at the onset of winter, would most likely be detrimental. Thus, if the fuel has the capability of altering the ecdysis process, it would have an indirect toxic effect on the arthropods. Further studies would be needed to assess model JP-5's effects on ecdysis.

2.3.5 Biodegradation of Model JP-5 Jet Fuel

An assessment of degradation of jet fuels by soil microbes was performed in culture flasks in order to determine the role of biota versus chemical and physical degradation in soil ecosystems. Model JP-5 jet fuel was used as a standard hydrocarbon mixture. Changes in known compounds and component quantities as a result of the microbial degradation were assessed by gas chromatographic techniques.

2.3.5.1 Procedure

Soil inoculum was prepared from 500 g of local soil and 1 L of chlorine-free, autoclaved tap water. The soil-water mixture was shaken and then permitted to stand until particles settled. The water was filtered through glass wool. Two tenths milliliter of this inoculum was added to 500 mL of basal medium in growth flasks. A standard sample of n-dodecylbenzene sulfonate sodium salt (DBSS) (Pfaltz and Bauer) at a concentration of 40.3 mg/mL was prepared. This is equivalent to 12.5 mg of carbon per 500 mL of medium. Standard DBSS (0.5 mL) and 0.2 mL of soil microbial inoculum were added to a growth flask (Flask A) containing 500 mL of basal medium. Model JP-5 (16.25 µL) was then added to 500 mL of basal medium, along with 0.2 mL of inoculum, in another growth flask (Flask B). Model JP-5 (130 µL) was added to a third flask (Flask C) containing 0.2 mL inoculum and 500 mL basal medium. Four adaptive transfers were made at two-day intervals; 1 mL of the contents of Flasks A, B, and C were transferred to 500 mL of fresh basal medium containing the same amounts of carbon sources as in the original flasks. A control (Flask D) was carried through with the positive carbon flasks.

Yeast extract was added to all growth flasks as a supplemental growth factor (1 mL/flask of 15 mg/100 mL). Table B-1 in Appendix B summarizes the experimental regimen. On the designated transfer days, all growth flasks were shaken vigorously by hand. Three milliliters of the contents of flasks A_1, A_2, A_3 and D were analyzed colorimetrically for DBSS content. Ten milliliters of the B and C series were extracted with 5 mL pentane in preparation for gas chromatography of model JP-5 (see chromatograms in Figures B-1 through B-8 and Table B-1).

2.3.5.2 Analytical Method

At each sampling time, 0.1 mL of each biodegradation culture was cultured on nutrient agar by adding it to the agar surface and streaking in three directions. The culture dishes were incubated at 37°C for 24 to 48 hours and then observed for relative amounts of colony growth and types of colonies.

Sampling, extraction, and analysis were performed sequentially over a 25-day period to analyze for indications of microbial degradation of the 14 components of our model JP-5 listed in Table B-2, Appendix B. The extraction procedure involved shaking 10 mL of medium from each fermentation flask with 5 mL of pentane. A 2-μL portion of this extract was analyzed by gas chromatography. The sequence of chromatograms obtained, including the chromatogram of standard JP-5, is shown in Figures B-1 through B-8.

The E_1 and E_2 series are duplicate studies of 16.25 μL model JP-5 added to 500 mL of basal medium; C_1 and C_2 are duplicate studies of 130 μL of model JP-5 added to 500 mL of basal medium.

2.3.5.3 Circular Profiling

The chromatograms reproduced in Appendix B show progressive, but many times very subtle, changes in the components of the model JP-5. Where very similar and complex sets of data are to be compared and such subtle differences noted, an optional plotting technique called circular profiling can aid in amplifying the differences. One data set, the C_1 series of the degradation study, was plotted using computerized circular profiling techniques [15].

The circular plots of the chromatograms are produced by entering retention times which relate these data to a scale factor selected to maximize the use of 360 degrees in a circular plot area. In order to maintain a consistent scale for comparison of the sequence of gas chromatograms, a factor of 4 was used as a scale factor since none of the retention times exceeded 90 minutes. The chromatograms illustrate the changes in the volatile components of JP-5 caused by microbial degradation. The corresponding peak area was plotted linearly along the radii emanating from the center of the circle at the appropriate number of degrees dictated by the retention times. To maximize the use of the plot area, the area percent data were normalized to the area percent value of the largest peak in any particular data set, then multiplied by the radius of the plot area. The distinctive pattern is produced by a

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[15] Ross, W. D., Hillan, W. J., Flayler, K. A., Pustinger, J. V., Brooks, J. J., and Eisentraut, K. J. Use of circular profiling techniques in gas chromatography. Journal of Chromatographic Science. 15:461, 1977.

line originating at the center of the circle, the origin, passing through each of the data points and finally returning to the origin.

Figures B-9 and B-10 in Appendix B illustrate the circular profiles with matching gas chromatograms for model JP-5 inoculated with soil microorganisms during the biodegradation investigations. The circular profiles readily attract attention to subtle changes in the gas chromatograms as the components change with time. This is especially true of the standard profile, day zero, day three and day five biodegradation curve (see Figure B-9).

2.3.5.4 Results

Major changes in the components of the model JP-5 took place in the 25 days of experimentation with concomitant increases in microbial growth curves. Peak height ratios changed, peak heights were reduced, and extraneous peaks appeared and disappeared with time. Ultimately, total peak areas were reduced dramatically. Microbial degradation appeared to play a lead role in these changes. This study has provided hydrocarbon transformation data obtained by isolating the effects of soil microbes in reaction flasks. Similar transformations of the hydrocarbons will be assessed in the soil ecosystem as the hydrocarbon compounds are transported through the soil.

2.4 ANALYTICAL METHODOLOGY

The two jet engine fuels used to evaluate the test procedures and to develop the terrestrial ecosystems were shale-derived JP-4, and a model JP-5 fuel that normally would contain equimolar amounts of 16 known compounds found in JP-5 fuel (see Table B-2). Two components *cis* and *trans*-decalin, were unavailable at the time the standard JP-5 was made, therefore, our model contained only 14 components. A similar model test mixture is also used by another contractor to study sorption of Air Force fuels by sediment. The Air Force interest is in determining the fate of these jet fuels in soil systems.

These mixtures of known hydrocarbon compounds can readily be gas chromatographed (see Figures 16, 17, and 18). Hence, the sample can be easily traced by gas chromatographic analyses as it progresses through the soil ecosystem.

2.4.1 Vertical Migration of Shale Derived JP-4 in Core Ecosystem

Test Core 10 was treated with 10 mL of shale-derived JP-4 by applying the fuel directly to the soil surface to mimic a spill of the material on a land surface. The 10-mL application is equivalent to a spill of 1.27 L/m^2. A biweekly application of 100 mL water was also used to simulate an equivalent of ~13 inches of rain per year estimated to mimic the amount of rainwater which would penetrate the soil in the Dayton, Ohio, area.

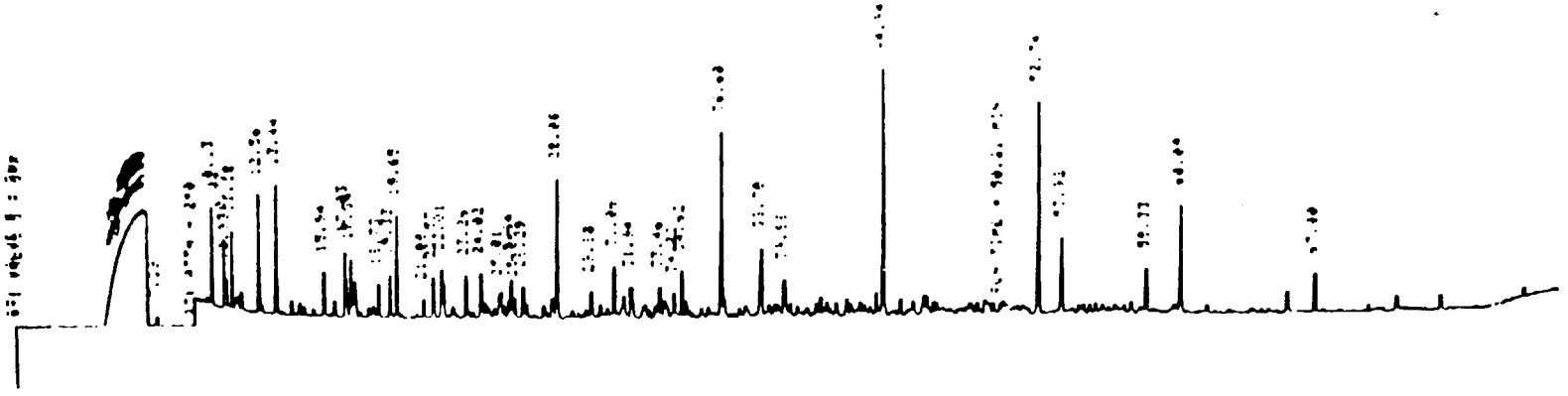

Figure 16. Gas chromatogram of JP-4 jet fuel.[a]

[a]Original gas chromatograph column used.

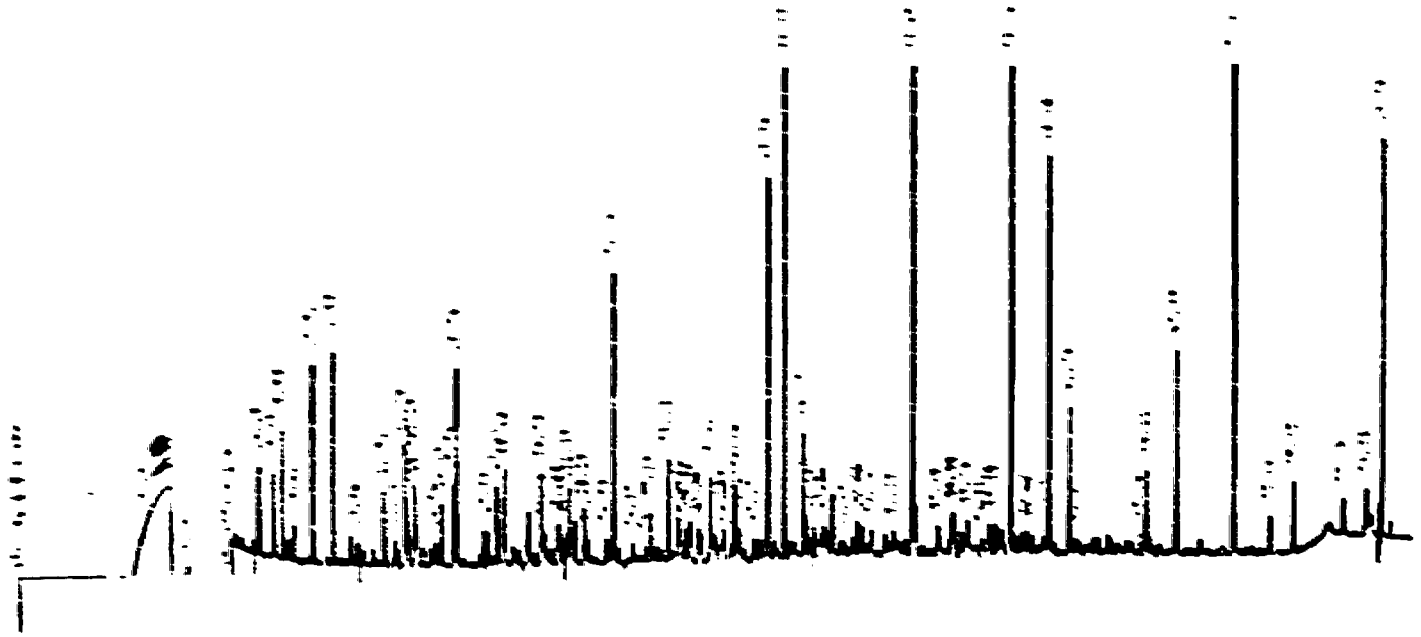

Figure 17. Gas Chromatogram of JP-4 jet fuel.[a]

[a]Retention times differ from the original due to a change in columns.

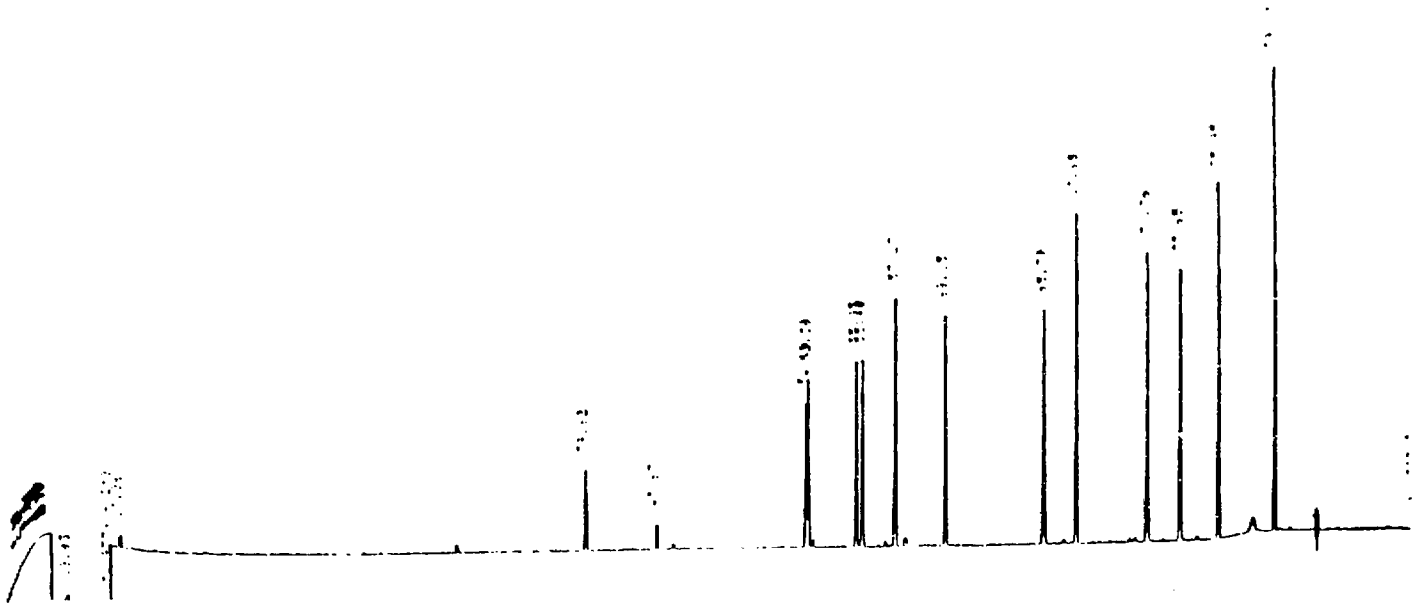

Figure 18. Gas chromatogram of standard model JP-5 jet fuel.

2.4.1.1 Results

n-Pentadecane and n-heptane are transported to a depth of 50 cm (bottom position in core). The boiling points of these two compounds vary the most of all the components in JP-4. Thus, transportation depth does not appear to be a phenomenon related to volatility. In addition, n-pentadecane and n-heptane were present in the soil for longer times than other compounds detected. n-Decane, n-undecane, n-tridecane, and n-tetradecane were only transported to a depth of 10 cm, and after 134 days into the experiment, they were no longer detected in the soil. n-Octane and n-nonane were not present at any depth, and it is concluded that neither is readily moved through the soil.

Nine hydrocarbons were identified in the JP-4 fuel and were followed throughout the experiment. The hydrocarbons are listed in Figure C-11 in Appendix C.

The quantities of specific hydrocarbon compounds and their residence time at each test depth (positions) in the soil core throughout the experiment time, is an assessment of the fate of components (or degradation products) of the jet fuels. Figures C-1 through C-10 in Appendix C are those chromatograms in which one or more of the hydrocarbons of interest was detected. Sampling Position 3 was the only position that did not show any hydrocarbons (as summarized in Appendix C, Table C-1). Figure C-11 in Appendix C indicates which of the hydrocarbons was detected, when, and where.

2.4.2 Movement of Model JP-5 in Indoor Core Ecosystem as Analyzed by Gas Chromatography

2.4.2.1 Description and Purpose

Cores 12 and 13 were used to (1) test the fate of model JP-5 in a soil core, and (2) compare field and laboratory data. Both core systems have five laterally located probes and a bottom outlet for sampling. Core 12 has probes located 6 cm apart on the horizontal axis and 10 cm apart vertically. The resulting effect is a spiral design. The positions are identified as 1 through 5 and bottom, the uppermost position being 1. Model JP-5 was tested in the laboratory in this core.

2.4.2.2 Procedure

Core 12 was dosed with 10 mL of model JP-5. A biweekly application of 100 mL of water was also used to simulate rainfall. Each sampling position, containing XAD-7 resin, was extracted with pentane as described in Section 2.4.1. Two microliters of the pentane extractions were analyzed by gas chromatography. Those chromatograms showing any of the 14 hydrocarbons present in our model JP-5 are presented in Figures D-1 through D-13 in Appendix D. Table D-1

in Appendix D summarizes when and where hydrocarbons are present and the number of different hydrocarbons detected.

2.4.2.3 Results

All the 14 hydrocarbons present in the model JP-5 were detected within the top 10 cm of the soil core. Six components, 1,3,5-trimethylbenzene, undecane, 1,2,4,5-tetramethylbenzene, 1,2,3,4-tetramethylbenzene, tetralin, and n-pentadecane, were transported to a 20-cm depth. Below the 20-cm depth only one component, 1,3,5-trimethylbenzene, was detected. This was detected at 50 cm on two separate occasions. Figure D-14, Appendix D, represents the above transport phenomenon which occurred in the laboratory core dosed with model JP-5.

2.4.3 Movement of Model JP-5 in Outdoor Core Ecosystem as Analyzed by Gas Chromatography

2.4.3.1 Description and Procedure

The outdoor ecosystem, Core 13, was subjected to the same test procedure as Core 12 with one exception; the core was not watered biweekly. Instead, it was exposed to natural rainfall and other weather-related conditions. As with Core 12, Core 13 had five side ports and one bottom port. The side ports, however, were not positioned in a spiral fashion as in Core 12. The probes were set in a straight vertical line 10 cm apart. This was necessary for accessability to the sampling probes from underground. The underground structure built for this field experiment allowed an individual to descend approximately six feet into the ground. The ground was held in place by wood panels on three sides. The fourth side was a stairway for passage. Adjacent to one side of the structure a hole was dug in the ground. The hole was big enough for the placement of the core ecosystem. The core ports were directed toward the wood panel. Holes were drilled through the panels to align with each port of the core. Teflon® tubing was used to attach the core probes to French squares located inside the underground structure. Following the same procedure described in Section 2.4.2.2, extracts were analyzed by gas chromatography. Those chromatograms indicating presence of one or more hydrocarbons are shown in Appendix E, Figures E-1 through E-21, and summarized in Appendix E, Table E-1.

2.4.3.2 Results

Appendix E, Figure E-22, charts the components of model JP-5 as they are transported through the outdoor core. The majority of the components were detected at a depth of 30 cm. Those components that were not detected at that depth are n-decane, undecane, dodecane, and n-tridecane. Of those four, n-tridecane and dodecane were not detected at any depth during the experiment. No hydrocarbons were transported below 30 cm. Comparing the fate of model

JP-5 in the laboratory core to the field core, the following similarities and differences were noted:

(1) XAD-7 resin in both cores proved to be a useful tool for the recovery of hydrocarbons in the ecosystems.

(2) The distance at which model JP-5 is transported in the indoor core is slightly greater (by 20 cm) than the outdoor core.

(3) The length of time over which components are detected is greater in the outdoor core than the indoor core.

(4) The selective transport of individual components as a function of time and depth varies.

The differences noted are minor in respect to the complexity of the system used. Differences in the degree of material movement can be theorized from channeling as a result of biota activity or water transport. However, the quantity of water leachate generated at a particular depth does not directly correlate with the hydrocarbon detection at that depth. Differences in the duration a component is detected in the soil column might be attributable to weather conditions, such as freezing temperatures.

2.4.4 Movement of Model JP-5 in Five, 7.5 Centimeter Diameter Cores as Analyzed by Gas Chromatography

A study was conducted for the assessment of several replicate core systems. The five cores used in this study were obtained in a polyvinyl chloride tube approximately 30 cm long and 7.5 cm in diameter. A 20-cm intact soil core was removed by the method described in Section 2.3.4.1. Each core was set up in the laboratory on a glass funnel with a stainless steel screen at the bottom end of the core. The stem of the funnel was connected to an adapter via a Teflon® tube; the adapter in turn screwed on to a French square bottle used for leachate collection. Each core was dosed with 6.5 mL of model JP-5 initially and with 6.5 mL water biweekly. XAD-7 was used to adsorb hydrocarbons as in previous studies. The resin in these cores was placed in the adapter. Prior to watering, the resin was removed and extracted with 5 mL of pentane. Two microliters of the pentane extract was injected into the gas chromatograph. Of the five dosed cores, two showed traces of hydrocarbons (see Appendix F, Figures F-1 through F-3).

2.4.5 Headspace Analysis of Shale-Derived JP-4 Dosed Cores by Gas Chromatography

Feasibility experiments were performed for the analysis of volatiles in the headspace of dosed cores by gas chromatography. With the use of a gas syringe, an injection was made using the headspace gas from a can containing shale-derived JP-4. A substantial number

of peaks were present on the chromatogram (see Appendix G, Figure G-1). Following positive results, injections involving the headspace of the core dosed with JP-4 (core 10) were performed. At seven days after dosing, 20 mL of gas was removed through the Teflon® septum (described in Section 2.1). As shown in Figure G-2, the chromatograph of this injection indicated few components in headspace; hence, little volatilization of jet fuel. After analyzing the previous results, a leak-tight syringe was substituted for the gas syringe. A chromatogram of a 10-mL injection of headgas removed by the leak-tight syringe is presented in Appendix G, Figure G-3.

Additional studies involved the use of a passive dosimeter for concentration of volatiles in the headspace. The passive dosimeter's design is based on diffusion principles and employs porous polymers as the sorbent element. This dosimeter has multi-organic vapor recovery capability and exhibits a sufficiently high collection rate to ensure monitoring at the ppb level.

Basically, the dosimeter consists of a stainless steel body 3.8 cm in diameter and 1.1 cm high. The internal diameter of the body is 3.5 cm with the exception of the central portion which is reduced to 3.0 cm I.D., to provide a precisely defined containment volume for the porous polymer. Stainless steel screens and perforated plates are stamped out with an appropriate dye and serve to confine the polymer within the dosimeter body and provide a diffusion barrier. Teflon® caps isolate the dosimeter from sample when not in use. The top cap is removed during sampling mode. Loading and assembly of the unit can be achieved within 2-3 minutes. The loaded dosimeter weighs approximately 36 g with a charge of ~0.4 g of Tenax GC polymer [16].

A passive dosimeter was suspended inside the core for several days after which it was removed for analysis. Although thermal desorption is normally used with the passive dosimeter, solvent extraction was used in these studies. Five milliliters of pentane was used to extract the hydrocarbons from the resin. One microliter of this extract was injected into the gas chromatograph. The resulting chromatogram is presented in Appendix G, Figure G-4.

2.4.5.1 Results

The passive dosimeter was chosen over a leak-tight syringe for monitoring headspace gases for two reasons. First, samples did not have to be analyzed immediately, which would have required

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[16] Wooten, G. W., and Strobel, J. E. Development of passive personal monitors for assessment of human exposure to toxic pollutants from energy sources. Quarterly Report No. 3, EPA Contract 68-02-3469. p. 2. August 1981.

that a gas chromatograph be immediately available each time a sample was taken. Second, the passive dosimeter concentrated the volatile hydrocarbons providing a more sensitive sampling technique.

2.4.6 Headspace Analysis of Model JP-5 Dosed Core by Gas Chromatography

Headspace analyses were performed on Cores 11 (control core) and 12 (JP-5-dosed laboratory core). Hydrocarbons were concentrated through the use of a passive dosimeter. Several resins and solvents were tested. Initial experiments involved the use of Tenax resin. Figures G-5 through G-9 in Appendix G are chromatograms resulting from those tests. Two solvents, pentane and methanol, were evaluated in the extraction procedures for separating the hydrocarbons from the resin. For each extraction, 5 mL of the solvent was used and 2 µL of the solvent extract was injected into the gas chromatograph. Both solvents were capable of extracting out the hydrocarbons. The other resin tested in the passive dosimeter mode was XAD-7. Again, with the use of pentane, hydrocarbons were recovered. Those chromatograms are presented in Figures G-10 through G-17 in Appendix G.

2.4.6.1 Results

Both of the solvents (pentane and methanol) and the resins (Tenax and XAD-7) proved to be acceptable for the use in headspace monitoring in core ecosystems. The resin and solvent chosen for this ecosystem study was XAD-7 and pentane, respectively. The decision was based on consistency with previous ecosystem work using that resin and solvent.

The passive dosimeter used with the control core picked up small amounts of hydrocarbons from the dosed core, even though the two cores were 30 cm (1 ft) apart.

2.4.7 Leachate Recovery in Core Ecosystems

A biweekly application of 100 mL of water was used to simulate an equivalent of ~13 inches of rain per year in cores 9, 10, 11 and 12. This volume of rainfall was selected by assuming about one-third of a 40-inch rainfall per year (western Ohio average rainfall) percolates into the soil while two-thirds evaporates or runs off. Core 13 was also watered with 100 mL of water biweekly while in the laboratory. Once equilibrium conditions were established, the core was moved outside, as described in Section 2.4.3.1. The top cap was removed from the core exposing the column to natural conditions. Thereafter, no further water was added. Similarly, no rainfall was simulated in field Core 14. All water incorporated into the system was the result of environmental conditions.

Leachates were recovered in French square bottles attached to the sampling probes by Teflon® tubing and volumes measured. The

leachate eluting from the bottom was also collected and measured. Appendix H, Tables H-1 through H-6, show the amounts of leachate recovered from probes and the bottom position of those ecosystems tested.

2.4.7.1 Results

The depth at which the water leachate flowed and its quantity varied from core to core. Within the same core there was also variaton from week to week, although the extent of variation was not as pronounced. These variations are explained by channeling effects caused by biota and/or physical stresses. Additionally, the quantity and depth of water leachate show no direct correlation with the quantities and depths of hydrocarbon transport.

2.4.8 Hydrazine Movement in Core Ecosystem

Because of highly reactive nature, hydrazine has been used in jet fuels as a source of energy. An effort was made to evaluate the fate of hydrazine in a field soil core ecosystem. Ten milliliters of 70% hydrazine was prepared and applied on day 113 to Core 14. Hydrazine was tested in the water leachate using HydraVer II[a] stabilizer and a colorimetric color disc. The test measured hydrazine from 0-1 mg/L. On day 140, the fourth position containing water leachate was analyzed for hydrazine. None was detected. On day 156 leachate from positions 2, 4 and the bottom were analyzed for hydrazine. Again, no hydrazine was detected. The fate of hydrazine in the core system is inconclusive in these studies. Metabolites and derivatives of hydrazine would not be detected with the test method employed and the possible reactivity of hydrazine with oxygen in the French square headspace could cause misleading data.

[a]Hach-formulated stable reagent.

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PERSONNEL INVOLVED IN THE RESEARCH EFFORT

W. D. Ross

J. M. Fullenkamp

W. J. Hillan

J. S. Noble

M. T. Wininger

J. A. Gridley

COMMUNICATIONS RELATED TO CONTRACT

Ross, W. D., J. M. Fullenkamp, W. J. Hillan, J. S. Noble, and M. T. Wininger. Environmental fate of jet fuels in soil ecosystems. Manuscript in preparation to be submitted to Environmental Science and Technology.

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A review of the contract status and research plans was presented by the principal investigator at the Second Annual Review of AFOSR Projects in San Antonio, Texas, January 1980.

A patent disclosure was initiated entitled "A laboratory terrestrial ecosystem for evaluating the fate and biological consequences of chemicals."

APPENDIX A

CYTOTOXICITY AND MUTAGENICITY DATA ON JET FUELS

TABLE A-1. CYTOTOXICITY DATA FOR SHALE JP-4 OBTAINED WITH CHO-K1 CELLS

CYTOTOXICITY DATA FOR SHALE JP-4(F33615)
CELL LINE: CHO-K1

8-18-81
PAGE REF: -

CONTROL (BACKGROUND) VALUES	MEAN VALUE	STANDARD DEVIATION
510	498	10
485		
504		
490		
500		

CONCENTRATION (UL/ML)	REPLICATE VALUES	MEAN VALUE	STANDARD DEVIATION	PERCENT SURVIVAL
5	0	0	0	0
	0			
	0			
1	0	0	0	0
	0			
	0			
.8	0	0	0	0
	0			
	0			
.4	14	8	7	2
	11			
	0			
.2	420	454	26	92
	479			
	460			
.1	498	488	15	100
	471			
	495			

BASIC
>

TABLE A-2. CYTOTOXICITY DATA FOR SHALE JP-4 OBTAINED WITH D98S CELLS

CYTOTOXICITY DATA FOR SHALE JP-4 (F33615)
CELL LINE: D98S

5-10-81
PAGE REF: -

CONTRO. (BACKGROUND) VALUES	MEAN VALUE	STANDARD DEVIATION
280 292 294 296 301	291	7

CONCENTRATION (UL/ML)	REPLICATE VALUES	MEAN VALUE	STANDARD DEVIATION	PERCENT SURVIVAL
2	0 0 0	0	0	0
1	0 0 0	0	0	0
.8	0 0 0	0	0	0
.6	0 0 20	17	29	6
.2	310 312 268	297	25	100
.1	287 297 315	300	14	100

BASIC
>

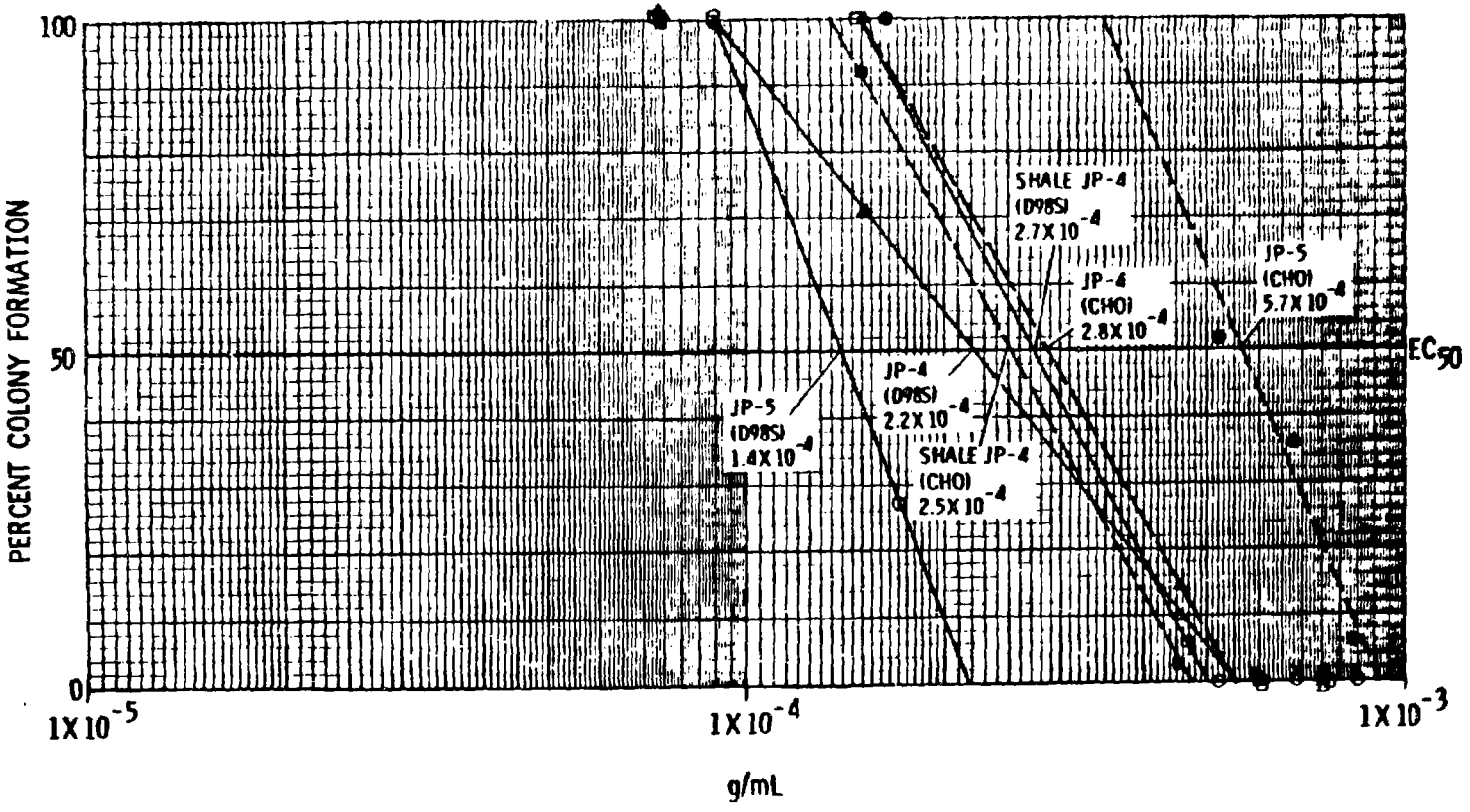

Figure A-1. Comparison of cytotoxicities of jet fuels to CHO (Chinese hamster) and D98S (human) cells.

TABLE A-3. CYTOTOXICITIES DETERMINED BY MAMMALIAN CELL CLONAL ASSAY

	CHO EC_{50}, g/mL	CHO EPA toxicity rating	D98S EC_{50}, g/mL	Whole animal* LD_{50}, mg/kg
Cadmium chloride	1.6×10^{-7}	high	2×10^{-7}	3 - 24
Potassium cyanide	4.8×10^{-7}	high	9×10^{-8}	4 - 16
Mercuric chloride	2.9×10^{-6}	high	5×10^{-7}	5 - 120
Hydroxylamine hydrochloride	3.2×10^{-5}	moderate	7×10^{-5}	100 - 400
Tetralin	5×10^{-5}	moderate	6×10^{-5}	-
Biphenyl	$>1 \times 10^{-4}$	low - very low	4×10^{-5}	56
Phenol	1.4×10^{-4}	low	1×10^{-5}	250 - 415
Ethyl benzene	1.7×10^{-4}	low	-	-
Shale oil-derived-JP-4	2.5×10^{-4}	low	2.7×10^{-4}	-
Petroleum-derived-JP-4	2.8×20^{-4}	low	2.2×10^{-4}	500
Chlorobenzene	5.5×10^{-4}	low	-	-
JP-5 (model)	5.7×10^{-4}	low	1.4×10^{-4}	-
Phenyl ether	6.5×10^{-4}	low	-	-
Aniline	7.1×10^{-4}	low	-	200 - 1250
Chloroform	1.4×10^{-2}	very low	-	700 - 1650

*Registry of Toxic Effects of Chemical Substances, Volumes I and II. R. J. Lewis, Sr., and Roger L. Tatken (eds). U.S. Department of Health and Human Services, NIOSH, September 1980.

COMPUTER ANALYSES OF AMES MUTAGENICITY ASSAY

SAMPLE ID:	LOG # 80-5-13-292-JP4 AFOSR			
STRAINS:	TA 98	TA100	TA 1535	TA1537
TEST DATES:	6/26/80	6/26/80	6/26/80	6/26/80
DATA REF:	1734156	NBF# 1734156	NBF#1734156	NBF#1734156
S-9 REF:	DA006 @ 10%			
SOLVENT	ETHANOL			

WITH MICROSOMAL ACTIVATION

REVERTANTS/PLATE

AMOUNT/PLATE(UL)	TA 98	TA100	TA 1535	TA1537
10	117 89 81	158 153 149	19 22 14	12 42 44
3	34 47 44	120 119 128	33 23 20	33 18 14
1	28 45 44	118 106 115	34 17 23	12 5 14
.2	27 35 30	186 145 138	17 27 27	2 10 7
.04	52 34 27	152 159 169	40 36 39	8 9 6
.01	43 34 15	188 163 170	29 41 30	5 7 11
CONTROLS				
SOLVENT	44 39 38	133 149 148	45 32 31[a]	10 6 13
NEGATIVE	27	165	23	17
POSITIVE	764	1642	357	111

WITH MICROSOMAL ACTIVATION

T VALUES

AMOUNT/PLATE(UL)	TA 98	TA100	TA 1535	TA1537
10	*3.64094	1.06477	-3.49558	2.48615
3	.107181	-2.45043	-1.85571	1.75769
1	-.230812	-3.69202	-2.08395	.055039
.2	-1.1847	1.23451	-2.20469	-1.27569
.04	-.443608	1.71619	.394958	-.438835
.01	-1.5499	*2.97702	-.385919	-.524381

* DENOTES A RESPONSE SIGNIFICANTLY DIFFERENT FROM CONTROLS AT P<0.01 LEVEL
DEGREES OF FREEDOM 14

[a]Solvent controls out of acceptable range. Requires retest.

LOG 80-5-13-292 JF4
TA98 WITH MICROSOMES
ORIGINAL TEST

DOSE LEVEL	REVERTANTS PER PLATE		
10.000	117.	85.	81.
3.0000	54.	47.	44.
1.0000	38.	45.	44.
.30000	37.	35.	30.
.40000E-01	33.	34.	27.
.10000E-01	41.	34.	15.
.0	44.	30.	38.

LOG 80-5-13-292 JF4
TA98 WITH MICROSOMES
ORIGINAL TEST

MEANS AND STANDARD DEVIATIONS OF LOGARITHMS OF PLATE COUNTS

DOSE LEVEL	LOG MEAN	LOG STD DEV	SAMPLE SIZE
10.000	1.9[illegible]	.8296E-01	3
3.0000	1.6[illegible]	.7421E-01	3
1.0000	1.5[illegible]	.1161	3
.30000	1.4[illegible]	.5448E-01	3
.40000E-01	1.5[illegible]	.1444	3
.10000E-01	1.447	.2401	3
.0	1.5[illegible]	.3997E-01	3

BARTLETT'S TEST RESULTS
F VALUE = 1.295
PROBABILITY LEVEL = .2437

THERE IS NO SIGNIFICANT DIFFERENCE AMONG TREATMENT VARIANCES

LOG 80-5-13-292 JF4
TA98 WITH MICROSOMES
ORIGINAL TEST

COMPARISON OF TREATMENTS WITH CONTROL (ONE-SIDED TEST)

DOSE LEVEL	T VALUE	PROB LEVEL
10.000	3.641	.1336E-02 **
3.0000	.1[illegible]	.4[illegible]
1.0000	-.2[illegible]	.4[illegible]
.30000	-1.1[illegible]	.1[illegible]
.40000E-01	-.44[illegible]	.3[illegible]
.10000E-01	-1.[illegible]	.7174E-01

NUMBER OF TREATMENTS SIGNIFICANTLY GREATER THAN CONTROL (P = 0.0100) = 1
THERE ARE NO TREATMENTS SIGNIFICANTLY SMALLER THAN THE CONTROL AT 0.0100 LEVEL

ERROR TERM FOR T TEST IS OBTAINED FROM POOLED WITHIN TREATMENTS VARIANCE OF .1554E-01 WITH 14 DEGREES OF FREEDOM

LOG 80-5-13-292 JF4
TA98 WITH MICROSOMES
ORIGINAL TEST
BASED ON 6 DOSE LEVELS AND 18 OBSERVATIONS
LOG COUNT = 1.447[illegible] + (.13081) LOG DOSE
CORRELATION COEFFICIENT = 0.6670
RESIDUAL STANDARD DEVIATION = 0.1616
T VALUE = 3.58
ONE-TAILED PROBABILITY LEVEL = 0.0012
DOSE-RESPONSE IS SIGNIFICANT AT 0.0100 LEVEL

F-VALUE FOR LACK-OF-FIT = 2.83
PROBABILITY LEVEL = 0.0729

LACK-OF-FIT IS NOT SIGNIFICANT

NO OUTLYING POINTS DETECTED IN RESIDUALS FROM LOG-LOG MODEL

STRAIN TA98 WITH MICROSOMES REQUIRES RETEST

1 TREATMENTS ARE SIGNIFICANTLY GREATER THAN THE CONTROL AT P = 0.0100

LOG 80-5-13-292 JF4

STRAIN TA98 WITH MICROSOMES REQUIRES RETEST

```
LOG 80-5-13-292  JF4
TA100  WITH MICROSOMES
ORIGINAL TEST

   DOSE LEVEL      REVERTANTS PER PLATE
   10.000            158.   153.   149.
   3.0000            120.   119.   126.
   1.0000            118.   106.   115.
   .20000            186.   145.   138.
   .40000E-01        152.   159.   169.
   .10000E-01        188.   163.   170.
   .0                133.   149.   148.

LOG 80-5-13-292  JF4
TA100  WITH MICROSOMES
ORIGINAL TEST

MEANS AND STANDARD DEVIATIONS OF LOGARITHMS OF PLATE COUNTS
   DOSE LEVEL      LOG MEAN   LOG STD DEV   SAMPLE SIZE
    10.00          2.184      .1276E-01          3
    3.000          2.087      .1733E-01          3
    1.000          2.053      .2431E-01          3
    .2000          2.180      .6948E-01          3
    .4000E-01      2.204      .2311E-01          3
    .1000E-01      2.239      .3184E-01          3
    .0             2.156      .2768E-01          3

BARTLETT TEST RESULTS
F RATIO              =     1.112
PROBABILITY LEVEL =     .3565

THERE IS NO SIGNIFICANT DIFFERENCE AMONG TREATMENT VARIANCES

LOG 80-5-13-292  JF4
TA100  WITH MICROSOMES
ORIGINAL TEST

COMPARISON OF TREATMENTS WITH CONTROL (ONE-SIDED TEST)

   DOSE LEVEL       T VALUE    PROB LEVEL
    10.00           1.065      .1525
    3.000          -2.450      .1401E-01 *
    1.000          -3.682      .1208E-02 **
    .2000           1.234      .1187
    .4000E-01       1.716      .5409E-01
    .1000E-01       2.977      .5000E-02 **

NUMBER OF TREATMENTS SIGNIFICANTLY GREATER THAN CONTROL   (P =  0.0100 ) =    1
NUMBER OF TREATMENTS SIGNIFICANTLY SMALLER THAN CONTROL (P =  0.0100 ) =    1
THESE TREATMENTS WILL BE OMITTED FROM DOSE-RESPONSE ANALYSIS

ERROR TERM FOR T TEST IS OBTAINED FROM POOLED WITHIN
TREATMENTS VARIANCE OF     .11707E-02 WITH  14 DEGREES OF FREEDOM

LOG 80-5-13-292  JF4
TA100  WITH MICROSOMES
ORIGINAL TEST
BASED ON  5 DOSE LEVELS AND  15 OBSERVATIONS
LOG COUNT =     2.1650     + (     -.30788E-01 ) LOG DOSE
CORRELATION COEFFICIENT      =       -0.5872
RESIDUAL STANDARD DEVIATION =        0.0512
T VALUE                      =         -2.62
ONE-SIDED PROBABILITY LEVEL =         0.5000
DOSE-RESPONSE IS NOT SIGNIFICANT SINCE SLOPE IS NEGATIVE

F-VALUE FOR LACK-OF-FIT =           4.97
PROBABILITY LEVEL       =         0.0231

LACK-OF-FIT IS NOT SIGNIFICANT

NO OUTLYING POINTS DETECTED IN RESIDUALS FROM LOG-LOG MODEL

STRAIN TA100      WITH MICROSOMES REQUIRES RETEST

1 TREATMENTS ARE SIGNIFICANTLY GREATER THAN THE CONTROL AT P =  0.0100

LOG 80-5-13-292  JF4

STRAIN TA100      WITH MICROSOMES REQUIRES RETEST
```

COMPUTER ANALYSES (continued)

TEST NUMBER	LOG#80-5-13-292 JP4
DATA REFERENCE	NRF#1734159
S-9 REFERENCE	10006
STRAIN	TA98
SOLVENT	ETHANOL
TEST DATE	7/9/80
POSITIVE CONTROLS	771
NEGATIVE CONTROLS	20

ANT PLATE (UL)	REVERTANTS/PLATE			T-VALUES	
CONTROL	28	22	21		
13	159	126	131	14.837	*
10	105	107	150	13.6	*
5	39	43	37	4.37825	*

* INDICATES A RESPONSE SIGNIFICANTLY DIFFERENT FROM CONTROLS AT THE P 0.01 LEVEL.
DEGREES OF FREEDOM = 8

COMPUTER ANALYSES (continued)

```
LOG 80-5-13-292  JP4
TA98   WITH MICROSOMES
RETEST
1 TREATMENTS SIGNIFICANTLY GREATER THAN CONTROL ON INITIAL TEST
AND DOSE-RESPONSE WAS ALSO SIGNIFICANT

   DOSE LEVEL      REVERTANTS PER PLATE
   13.000            159.   126.   131.
   10.000            105.   107.   150.
   5.0000             39.    43.    37.
   .0                 28.    22.    21.

LOG 80-5-13-292  JP4
TA98   WITH MICROSOMES
RETEST
1 TREATMENTS SIGNIFICANTLY GREATER THAN CONTROL ON INITIAL TEST
AND DOSE-RESPONSE WAS ALSO SIGNIFICANT

MEANS AND STANDARD DEVIATIONS OF LOGARITHMS OF PLATE COUNTS
   DOSE LEVEL      LOG MEAN   LOG STD DEV   SAMPLE SIZE
    13.00          2.140       .5411E-01          3
    10.00          2.076       .8716E-01          3
    5.000          1.598       .3312E-01          3
    .0             1.371       .6707E-01          3

BARTLETTS TEST RESULTS
F RATIO             =     .4789
PROBABILITY LEVEL   =     .6976

THERE IS NO SIGNIFICANT DIFFERENCE AMONG TREATMENT VARIANCES

LOG 80-5-13-292  JP4
TA98   WITH MICROSOMES
RETEST
1 TREATMENTS SIGNIFICANTLY GREATER THAN CONTROL ON INITIAL TEST
AND DOSE-RESPONSE WAS ALSO SIGNIFICANT

COMPARISON OF TREATMENTS WITH CONTROL (ONE-SIDED TEST)

   DOSE LEVEL       T VALUE    PROB LEVEL
    13.00           14.84       .2862E-06 ***
    10.00           13.60       .5599E-06 ***
    5.000            4.379      .1176E-02 **

NUMBER OF TREATMENTS SIGNIFICANTLY GREATER THAN CONTROL   (P =  0.0100 ) =   3
THERE ARE NO TREATMENTS SIGNIFICANTLY SMALLER THAN THE CONTROL AT  0.0100 LEVEL

ERROR TERM FOR T TEST IS OBTAINED FROM POOLED WITHIN
TREATMENTS VARIANCE OF     .40301E-02 WITH   8 DEGREES OF FREEDOM

LOG 80-5-13-292  JP4
TA98   WITH MICROSOMES
RETEST
1 TREATMENTS SIGNIFICANTLY GREATER THAN CONTROL ON INITIAL TEST
AND DOSE-RESPONSE WAS ALSO SIGNIFICANT
BASED ON  3 DOSE LEVELS AND   9 OBSERVATIONS
LOG COUNT =      .65883      + (      1.3638      ) LOG DOSE
CORRELATION COEFFICIENT     =        0.9660
RESIDUAL STANDARD DEVIATION =        0.0724
T VALUE                     =          9.89
ONE-SIDED PROBABILITY LEVEL =        0.0000
DOSE-RESPONSE IS SIGNIFICANT AT  0.0100 LEVEL

F-VALUE FOR LACK-OF-FIT =          3.47
PROBABILITY LEVEL       =        0.1117

LACK-OF-FIT IS NOT SIGNIFICANT

NO OUTLYING POINTS DETECTED IN RESIDUALS FROM LOG-LOG MODEL


STRAIN TA98       WITH MICROSOMES IS POSITIVE

4 TREATMENTS ARE SIGNIFICANTLY GREATER THAN THE CONTROL AT P =  0.0100
AND DOSE-RESPONSE IS SIGNIFICANT AT P =  0.0100

LOG 80-5-13-292  JP4


STRAIN TA98       WITH MICROSOMES IS POSITIVE


AMES TEST IS COMPLETED . COMPOUND IS POSITIVE .
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COMPUTER ANALYSES (continued)

TEST NUMBER	LOG#80-5-13-292 JFA
DATA REFERENCE	NBF#1734159
S-9 REFERENCE	DA006 @10%
STRAIN	TA100 W/ S-9
SOLVENT	ETHANOL
TEST DATE	7/9/80
POSITIVE CONTROLS	1447
NEGATIVE CONTROLS	150

AMT/PLATE (UL)	REVERTANTS/PLATE			T-VALUES
CONTROL	148	194	144	
.013	136	167	134	-.933091
.01	134	184	155	-.194497
.001	155	182	164	.342351

* INDICATES A RESPONSE SIGNIFICANTLY DIFFERENT FROM CONTROLS AT THE P 0.01 LEVEL.
DEGREES OF FREEDOM 7

LOG 80-5-13-292 JP4
TA100 WITH MICROSOMES
RETEST
1 TREATMENTS SIGNIFICANTLY GREATER THAN CONTROL ON INITIAL TEST
BUT DOSE-RESPONSE WAS NOT SIGNIFICANT

DOSE LEVEL	REVERTANTS PER PLATE		
.1300E-01	136.	167.	134.
.1000E-01	136.	184.	155.
.5000E-02	155.	182.	164.
.0	148.	194.	144.

LOG 80-5-13-292 JP4
TA100 WITH MICROSOMES
RETEST
1 TREATMENTS SIGNIFICANTLY GREATER THAN CONTROL ON INITIAL TEST
BUT DOSE-RESPONSE WAS NOT SIGNIFICANT

MEANS AND STANDARD DEVIATIONS OF LOGARITHMS OF PLATE COUNTS

DOSE LEVEL	LOG MEAN	LOG STD DEV	SAMPLE SIZE
.1300E-01	2.161	.5344E-01	3
.1000E-01	2.196	.6584E-01	3
.5000E-02	2.209	.3538E-01	3
.0	2.205	.7154E-01	3

BARTLETTS TEST RESULTS
F RATIO = .2771
PROBABILITY LEVEL = .8418

THERE IS NO SIGNIFICANT DIFFERENCE AMONG TREATMENT VARIANCES

LOG 80-5-13-292 JP4
TA100 WITH MICROSOMES
RETEST
1 TREATMENTS SIGNIFICANTLY GREATER THAN CONTROL ON INITIAL TEST
BUT DOSE-RESPONSE WAS NOT SIGNIFICANT

COMPARISON OF TREATMENTS WITH CONTROL (ONE-SIDED TEST)

DOSE LEVEL	T VALUE	PROB LEVEL
.1300E-01	-.9330	.1891
.1000E-01	-.1945	.4253
.5000E-02	.3423	.3705

THERE ARE NO TREATMENTS SIGNIFICANTLY GREATER THAN THE CONTROL AT 0.0100 LEVEL
THERE ARE NO TREATMENTS SIGNIFICANTLY SMALLER THAN THE CONTROL AT 0.0100 LEVEL

ERROR TERM FOR T TEST IS OBTAINED FROM POOLED WITHIN
TREATMENTS VARIANCE OF .33902E-02 WITH 6 DEGREES OF FREEDOM

LOG 80-5-13-292 JP4
TA100 WITH MICROSOMES
RETEST
1 TREATMENTS SIGNIFICANTLY GREATER THAN CONTROL ON INITIAL TEST
BUT DOSE-RESPONSE WAS NOT SIGNIFICANT
BASED ON 3 DOSE LEVELS AND 9 OBSERVATIONS
LOG COUNT = 1.9175 + (-.13359) LOG DOSE
CORRELATION COEFFICIENT = -0.4683
RESIDUAL STANDARD DEVIATION = 0.0500
T VALUE = -1.40
ONE-SIDED PROBABILITY LEVEL = 0.5000
DOSE-RESPONSE IS NOT SIGNIFICANT SINCE SLOPE IS NEGATIVE

F-VALUE FOR LACK-OF-FIT = 0.23
PROBABILITY LEVEL = 0.6495

LACK-OF-FIT IS NOT SIGNIFICANT

NO OUTLYING POINTS DETECTED IN RESIDUALS FROM LOG-LOG MODEL

STRAIN TA100 WITH MICROSOMES IS NEGATIVE

LOG 80-5-13-292 JP4

STRAIN TA100 WITH MICROSOMES IS NEGATIVE

COMPUTER ANALYSES (continued)

TEST MATERIAL	LOG #00-5-13-292 JR4
DATA REFERENCE	NPP01234159
S-9 REFERENCE	10000 0107
STRAIN	TA1535 W/ S-9
SOLVENT	ETHANOL
TEST DATE	7/9/00
POSITIVE CONTROLS	240
NEGATIVE CONTROLS	16

ANT/PLATE (UL)	REVERTANTS/PLATE			T-VALUES
CONTROL	19	11	16	
10	31	19	27	1.25447
3	16	25	17	-.222014
1	14	10	12	-1.54297
.3	10	16	0	-1.11042
.04	15	15	15	-.507685
.01	12	22	12	-.674354

* INDICATES A MEANING SIGNIFICANTLY DIFFERENT FROM CONTROLS AT THE 0.05 LEVEL.
DEGREES OF FREEDOM = 14

COMPUTER ANALYSES (continued)

SAMPLE ID:	LOG # 80-5-13-292-JP4 AFOSR			
STRAINS:	TA98	TA100	TA1535	TA1537
TEST DATES:	6/26/80	6/26/80	6/26/80	6/26/80
DATA REF:	NBF#1734156	NBF#1734156	NBF#1734156	NBF#1734156
S-9 REF:	NONE			
SOLVENT	ETHANOL 20UL			

WITHOUT MICROSOMAL ACTIVATION

REVERTANTS/PLATE

AMOUNT/PLATE(UL)	TA98			TA100			TA1535			TA1537		
10	23	42	17	160	159	160	27	34	26	21	17	25
3	27	17	30	115	117	147	19	20	16	16	13	9
1	32	24	37	128	94	106	24	29	15	8	15	9
.2	27	19	12	146	108	95	26	18	19	13	6	5
.04	17	24	18	126	113	100	31	25	24	5	14	5
.01	19	11	22	93	124	115	23	29	34	5	3	4
CONTROLS												
SOLVENT	16	21	17	130	111	129	29	28	24	4	8	7
NEGATIVE	19			161			41			11		
POSITIVE		964			767			479			543	

WITHOUT MICROSOMAL ACTIVATION

T VALUES

AMOUNT/PLATE(UL)	TA98	TA100	TA1535	TA1537
10	1.35836	2.29802	.431903	*3.89285
3	1.13181	.178185	-2.48141	2.24289
1	2.06365	-1.11061	-1.32967	1.66181
.2	9.62859E-02	-.638154	-1.67157	.585782
.04	.323254	-.787294	-9.93526E-02	.471463
.01	-.279052	-.995917	.322948	-1.39162

* DENOTES A RESPONSE SIGNIFICANTLY DIFFERENT FROM CONTROLS AT P<0.01 LEVEL
DEGREES OF FREEDOM 14

LOG 80-5-13-292 JP4
TA1537 WITHOUT MICROSOMES
ORIGINAL TEST

DOSE LEVEL	REVERTANTS PER PLATE		
10.000	21.	17.	25.
3.0000	16.	13.	9.
1.0000	8.	15.	6.
.30000	13.	6.	5.
.40000E-01	5.	14.	5.
.10000E-01	5.	3.	4.
.0	4.	8.	7.

LOG 80-5-13-292 JP4
TA1537 WITHOUT MICROSOMES
ORIGINAL TEST

MEANS AND STANDARD DEVIATIONS OF LOGARITHMS OF PLATE COUNTS

DOSE LEVEL	LOG MEAN	LOG STD DEV	SAMPLE SIZE
10.00	1.317	.8387E-01	3
3.000	1.091	.1265	3
1.000	1.011	.1451	3
.3000	.8214	.2503	3
.4000E-01	.6480	.2382	3
.1000E-01	.5917	.1113	3
.0	.7834	.1507	3

BARTLETT'S TEST RESULTS
F RATIO = .4365
PROBABILITY LEVEL = .8181

THERE IS NO SIGNIFICANT DIFFERENCE AMONG TREATMENT VARIANCES

LOG 80-5-13-292 JP4
TA1537 WITHOUT MICROSOMES
ORIGINAL TEST

COMPARISON OF TREATMENTS WITH CONTROL (ONE-SIDED TEST)

DOSE LEVEL	T VALUE	PROB LEVEL	
10.00	3.893	.8122E-03	***
3.000	2.243	.2080E-01	*
1.000	1.642	.5939E-01	
.3000	.6863	.2887	
.4000E-01	.4715	.3223	
.1000E-01	-1.392	.9288E-01	

NUMBER OF TREATMENTS SIGNIFICANTLY GREATER THAN CONTROL (P = 0.0100) = 1
THERE ARE NO TREATMENTS SIGNIFICANTLY SMALLER THAN THE CONTROL AT 0.0100 LEVEL

ERROR TERM FOR T TEST IS OBTAINED FROM POOLED WITHIN
TREATMENTS VARIANCE OF .2816BE-01 WITH 14 DEGREES OF FREEDOM

LOG 80-5-13-292 JP4
TA1537 WITHOUT MICROSOMES
ORIGINAL TEST
BASED ON 4 DOSE LEVELS AND 18 OBSERVATIONS
LOG COUNT = 1.0450 + (.20863) LOG DOSE
CORRELATION COEFFICIENT = 0.8244
RESIDUAL STANDARD DEVIATION = 0.1585
T VALUE = 5.83
ONE-SIDED PROBABILITY LEVEL = 0.0000
DOSE-RESPONSE IS SIGNIFICANT AT 0.0100 LEVEL

F-VALUE FOR LACK-OF-FIT = 0.51
PROBABILITY LEVEL = 0.7291

LACK-OF-FIT IS NOT SIGNIFICANT

NO OUTLYING POINTS DETECTED IN RESIDUALS FROM LOG-LOG MODEL

STRAIN TA1537 WITHOUT MICROSOMES REQUIRES RETEST

1 TREATMENTS ARE SIGNIFICANTLY GREATER THAN THE CONTROL AT P = 0.0100

LOG 80-5-13-292 JP4

STRAIN TA1537 WITHOUT MICROSOMES REQUIRES RETEST

APPENDIX B

DEGRADATION OF MODEL JI-5

B, Series

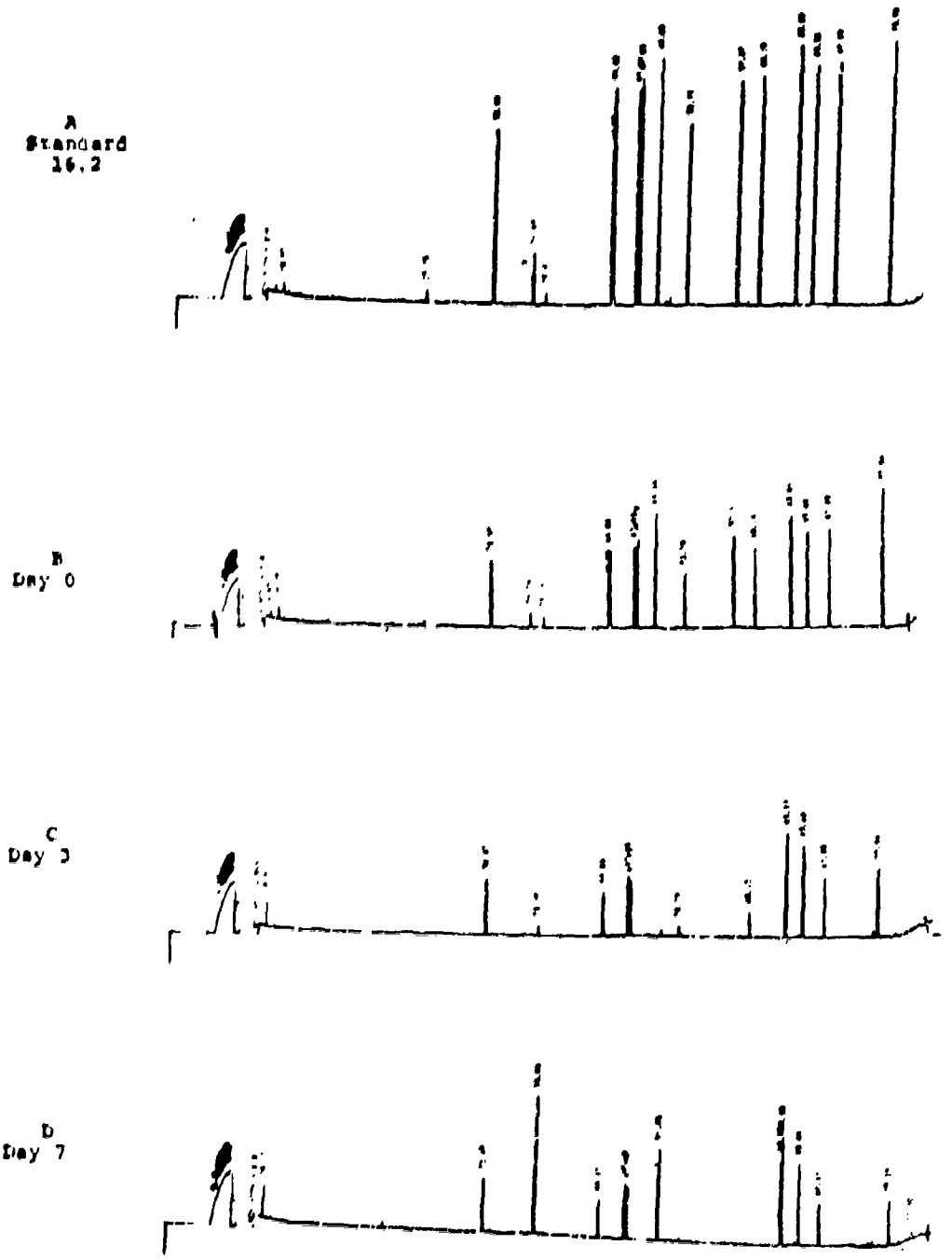

Figure B-1. Gas chromatogram of pentane extracts from biodegradation of model JP-5, 16.25 µL-standard, days 0, 3, 7.

B, Series (continued)

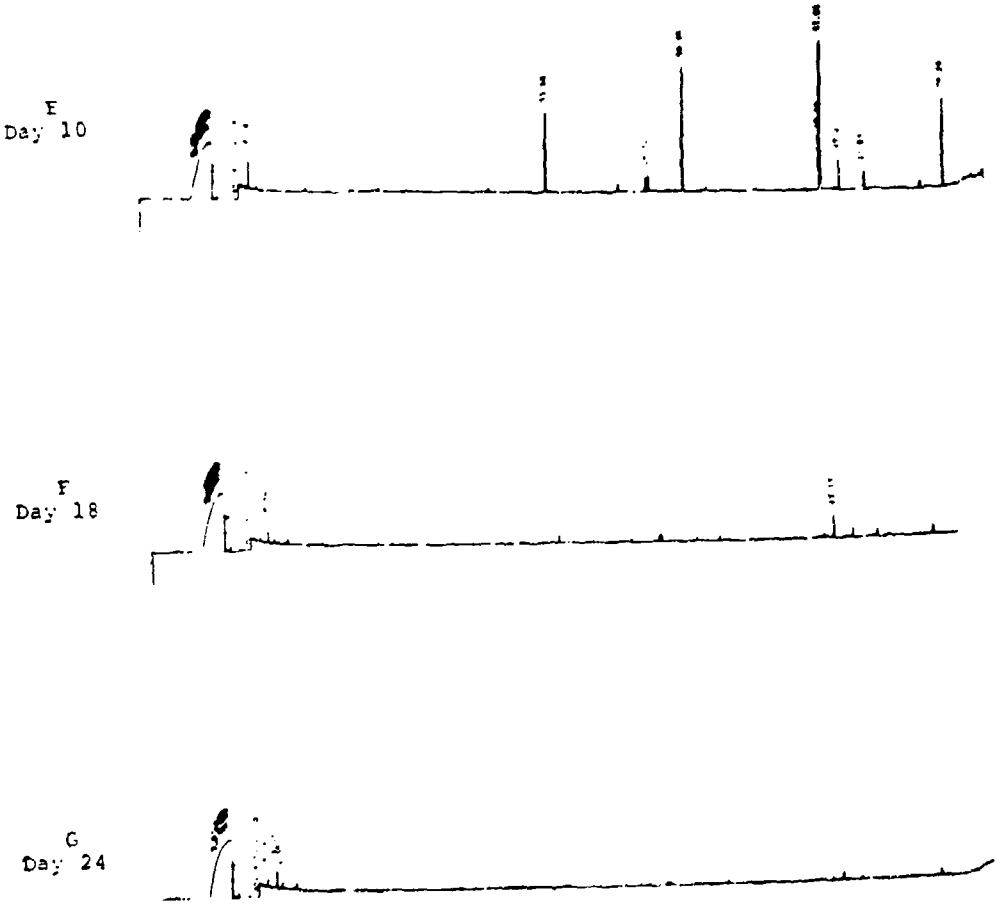

Figure B-2. Gas chromatogram of pentane extracts from biodegradation of model JP-5, B_1-16.25 µL-standard, day 10, 18, 24.

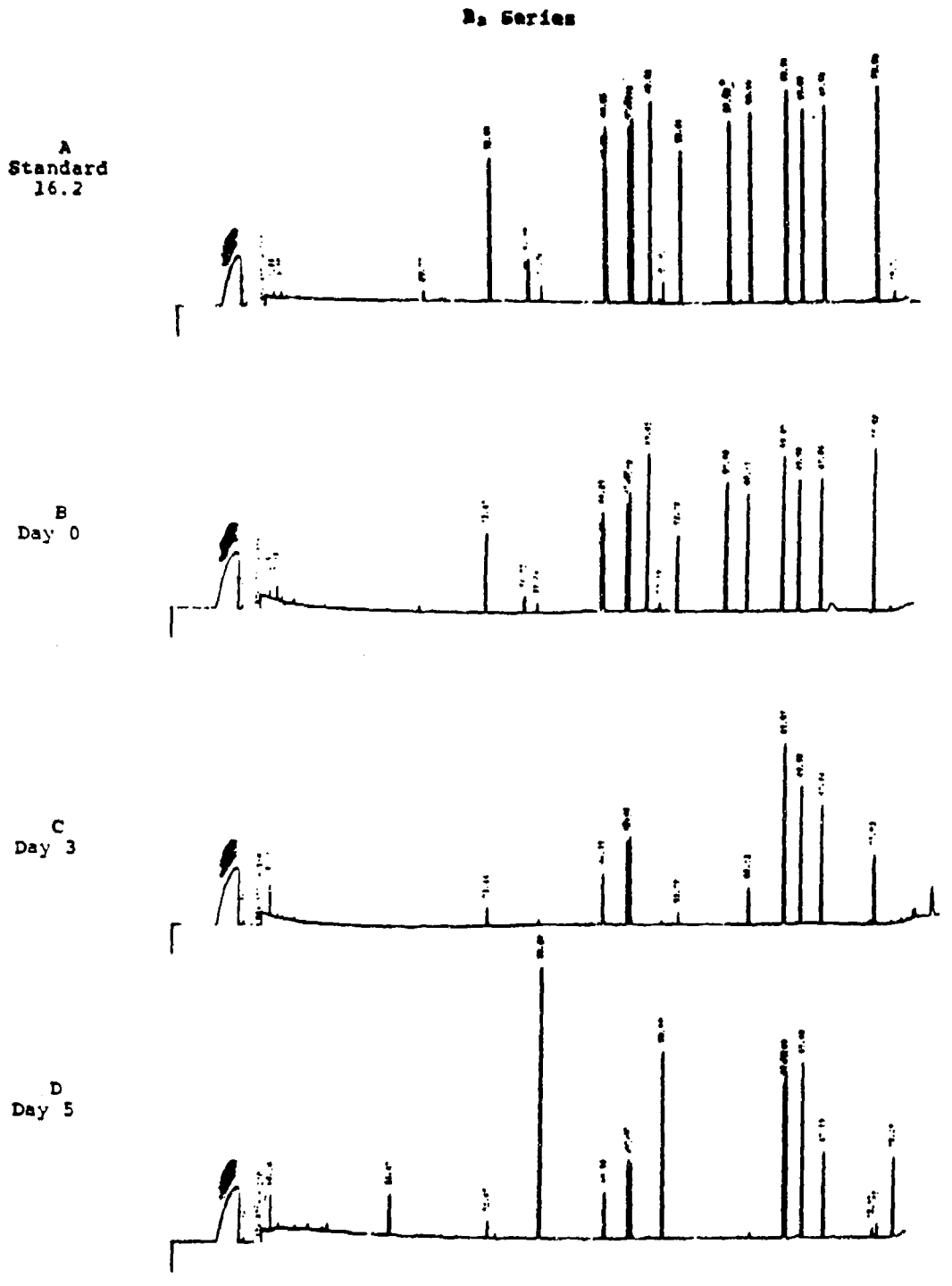

Figure B-3. Gas chromatogram of pentane extracts from biodegradation of model JP-5, B_2-16.25 µL-standard, day 0, 3, 5.

B_2 Series (continued)

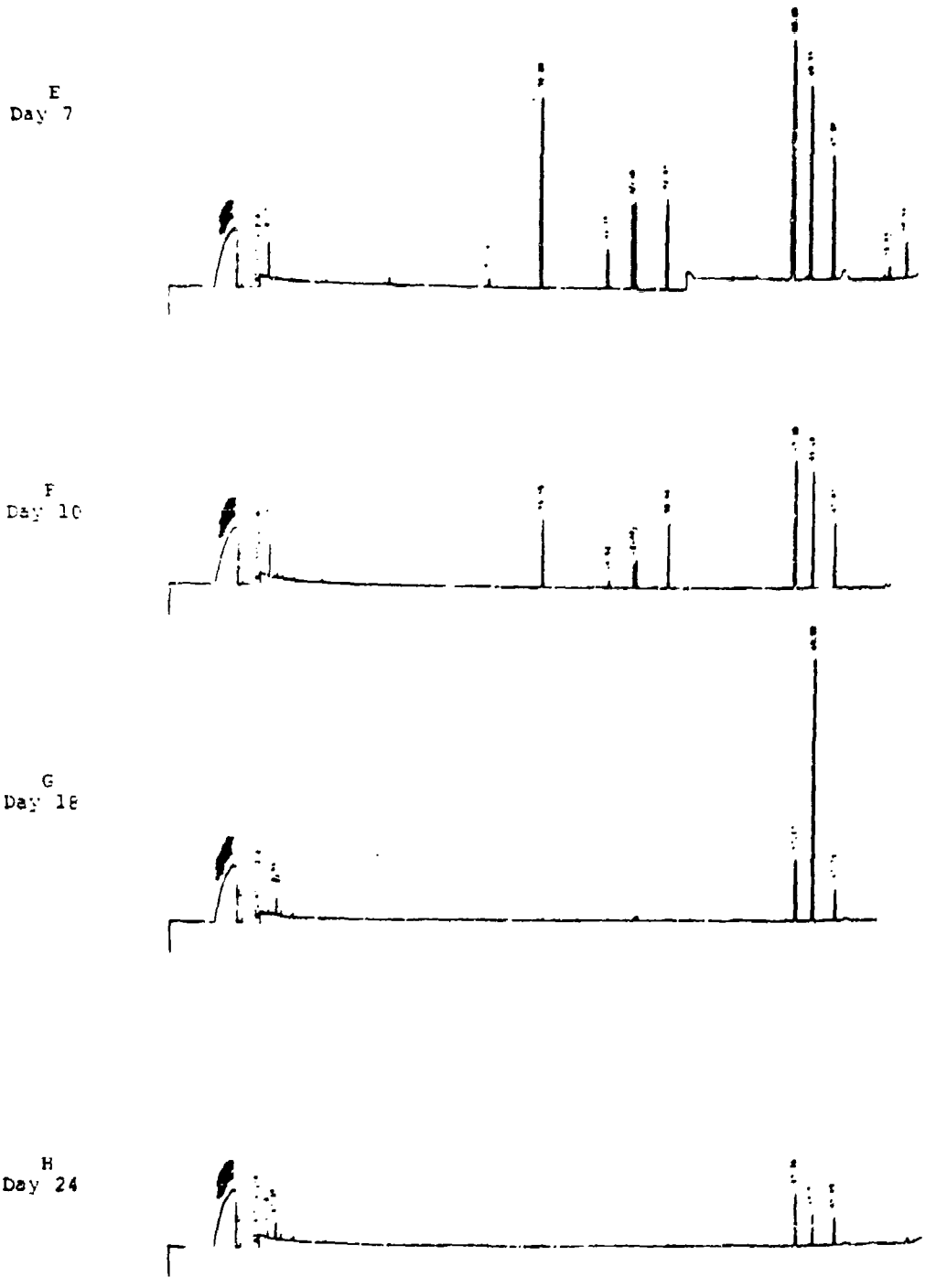

Figure B-4. Gas chromatogram of pentane extracts from biodegradation of model JP-5, B_2-16.25 µL-standard, day 7, 10, 18, 24.

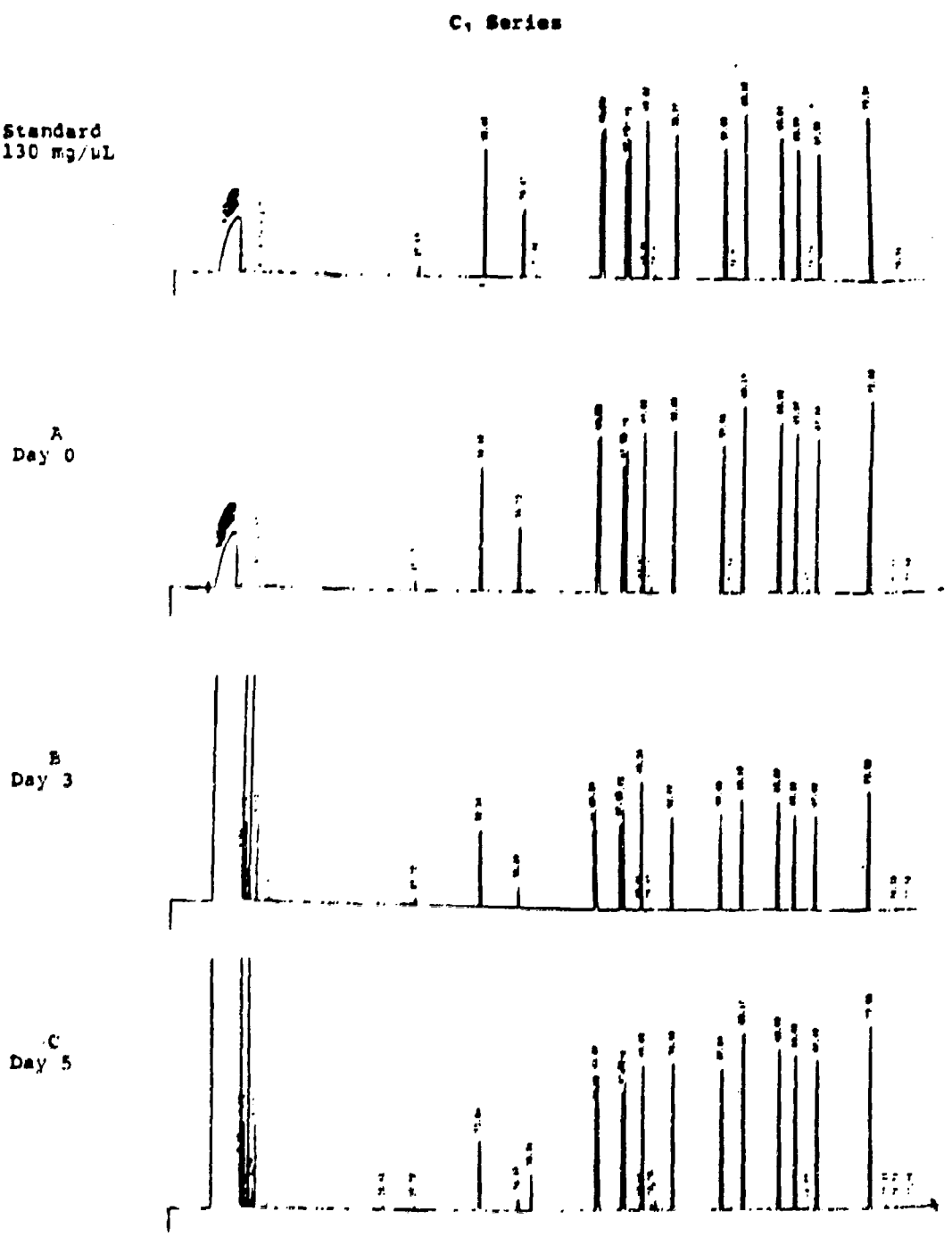

Figure B-5. Gas chromatogram of pentane extracts from biodegradation of model JP-5, C_1-130 µL-standard, day 0, 3, 5.

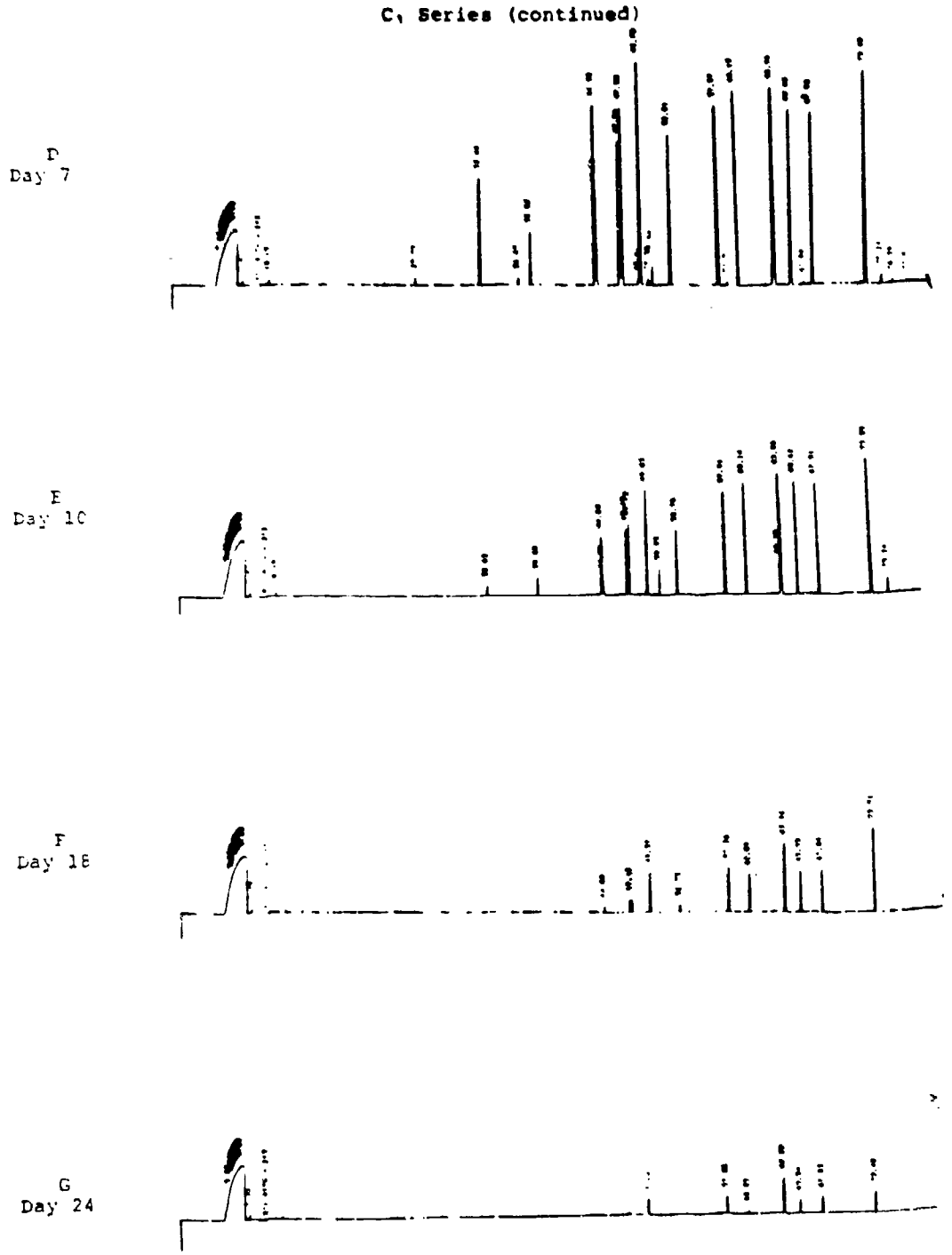

Figure B-6. Gas chromatogram of pentane extracts from biodegradation of model JP-5, C_1-130 µL-standard, day 7, 10, 18, 24.

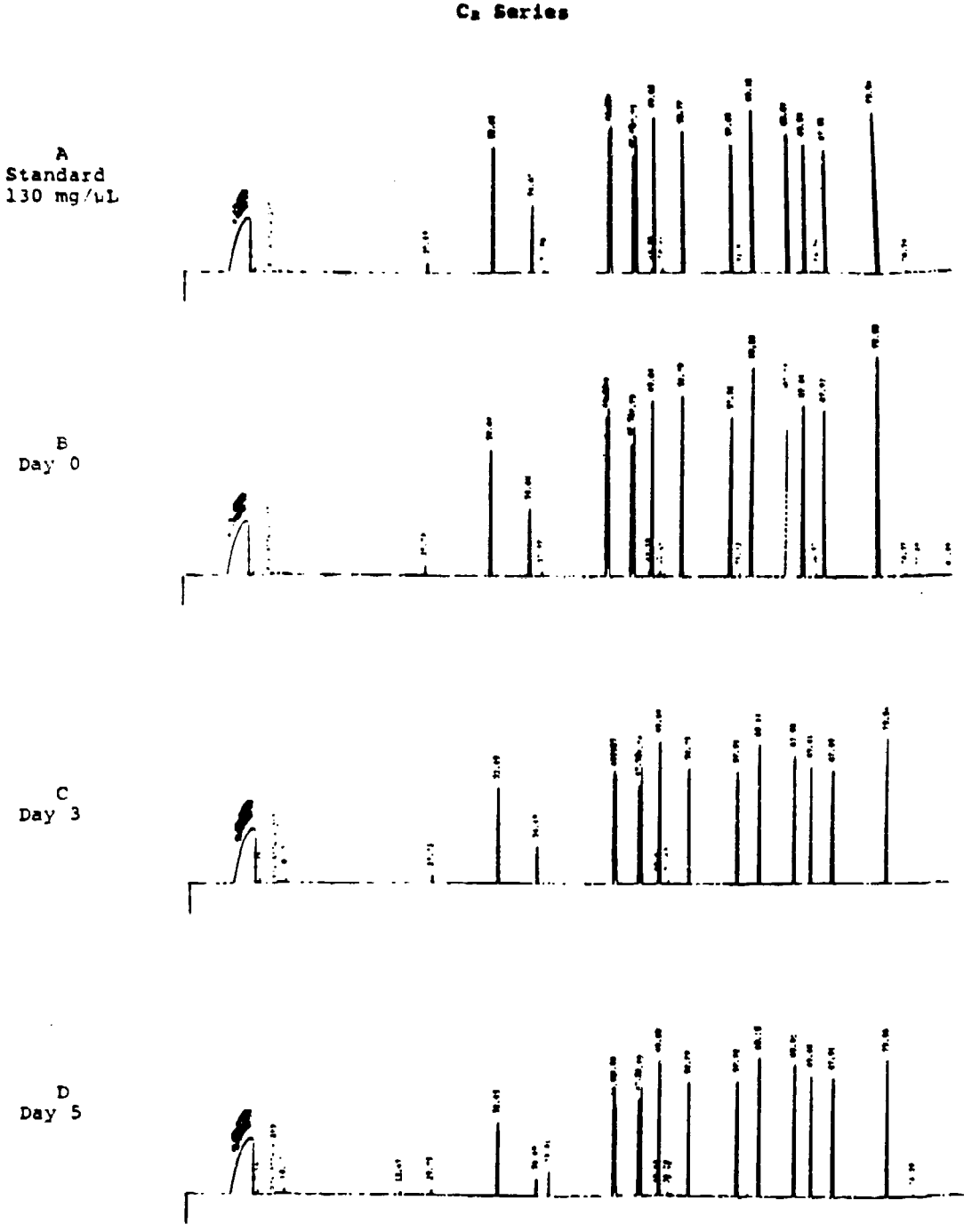

Figure B-7. Gas chromatogram of pentane extracts from biodegradation of model JP-5 C_2-130 µL-standard, day 0, 3, 5.

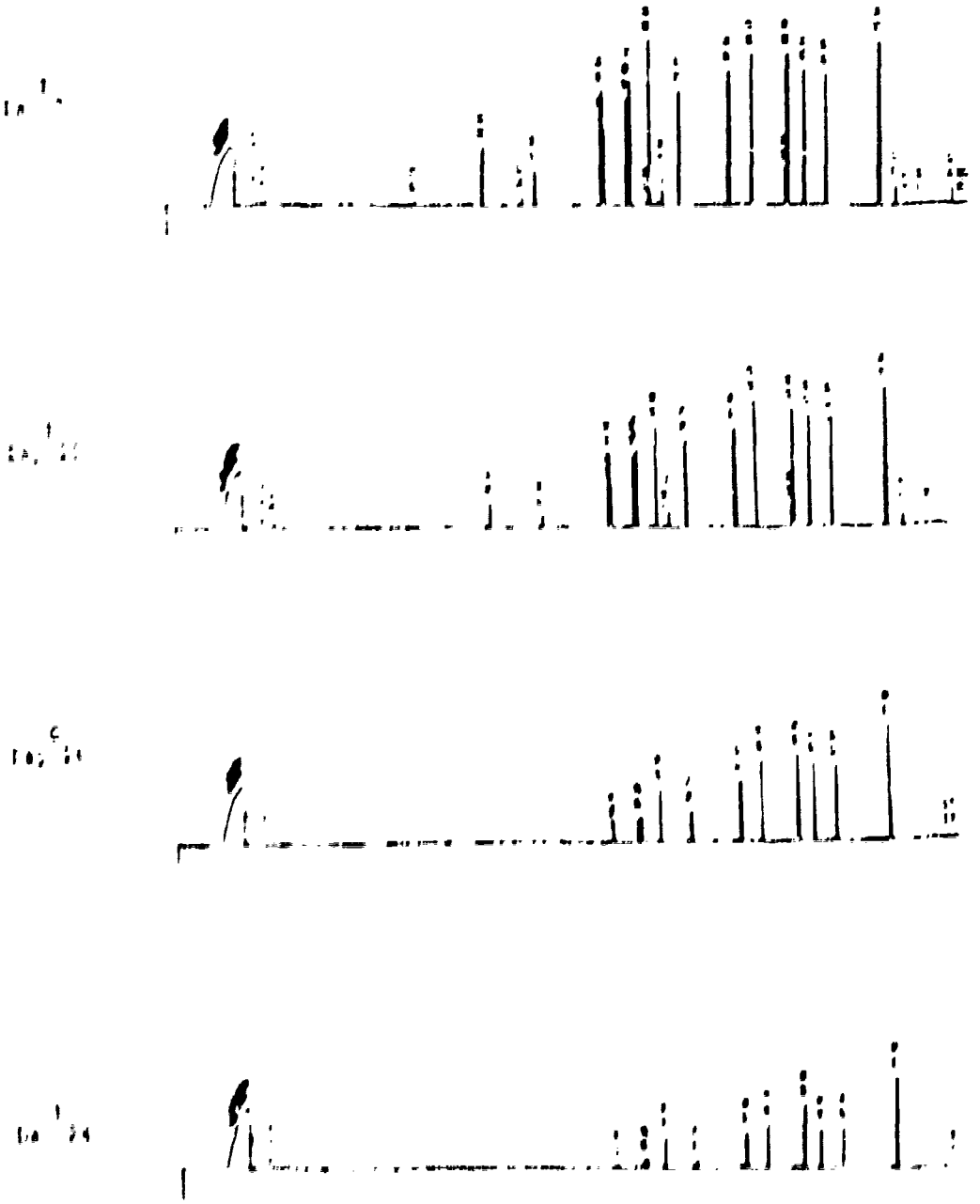

Figure I-6. Gas chromatogram of pentane extracts from biodegradation of model JP-5 C_2-130 pl- standard, day 7, 10, 16, 24.

TABLE B-1. EXPERIMENTAL PROCEDURE FOR MODEL JP-5 BIODEGRADATION STUDY

Flask	Basal medium, 500 mL	Yeast extract, 1.0 mL	Carbon source	Inoculum
A_1	+	+	0.5 mL DBSS stock* (12.5 mg C)	1.0 mL acclimated Flask A
A_2	+	+	0.5 mL DBSS stock* (12.5 mg C)	1.0 mL acclimated Flask A
A_3	+	+	0.5 mL DBSS stock* (12.5 mg C)	None
B_1	+	+	16.25 µL JP-5 (12.5 mg C)	1.0 mL acclimated Flask B
B_2	+	+	16.25 µL JP-5 (12.5 mg C)	1.0 mL acclimated Flask B
B_3	+	+	16.25 µL JP-5 (12.5 mg C)	None
C_1	+	+	130 µL JP-5 (100 mg C)	1.0 mL acclimated Flask C
C_2	+	+	130 µL JP-5 (100 mg C)	1.0 mL acclimated Flask C
C_3	+	+	130 µL JP-5 (100 mg C)	None
D	+	+	None	0.33 mL acclimated cells from A, B, and C Flasks

*DBSS Stock - Dodecylbenzene sodium sulfonate (standard) (40.3 mg/mL) - (25 mg carbon/mL)

TABLE B-2. MODEL JP-5 COMPONENTS

Name	Empirical formula	Molecular weight	Boiling point, °C	Melting point, °C	Density, d$_4^{20}$	Amount added
1,3,5-Trimethylbenzene	C_9H_{12}	120.19	164 - 165	-	0.8637	13.9 mL[a]
n-Decane	$C_{10}H_{22}$	142.28	174	-	0.7300	10.0 mL[a]
Cyclooctane	$C_{16}H_{32}$	138	194.7	-	0.8453	15.4 mL[a]
Hydrocarbon	$C_{16}H_{32}$	138	187	-	0.87	15.9 mL
n-Undecane	$C_{11}H_{24}$	156	196	-	0.74017	21.0 mL
1,2,4,5-Tetramethylbenzene	$C_{10}H_{14}$	134	-	80 - 82	0.84[b]	16.0 g
1,2,3,5-Tetramethylbenzene	$C_{10}H_{14}$	134	204 - 205	-	0.861	14.0 mL
1,2,3,4-Tetrahydronaphthalene	$C_{10}H_{12}$	132	207		0.9702	13.6 mL
Naphthalene	$C_{10}H_8$	128		78 - 81	1.162	12.8 g
n-Dodecane	$C_{12}H_{26}$	170	215		0.7487	22.7 mL
2-Methylnaphthalene	$C_{11}H_{10}$	142		32 - 34	1.029	13.8 mL
Acenaphthene	$C_{12}H_{10}$	154	278		0.7559	24.2 mL
Tridecane	$C_{13}H_{28}$	184	234	66 - 71	1.041	14.8 mL
Biphenyl	$C_{12}H_{10}$	154	254	109 - 110	1.008	15.6 g
2,6-Dimethylnaphthalene	$C_{12}H_{12}$	156				
n-Pentadecane	$C_{15}H_{32}$	212	270		0.7568	27.6 mL
1,3-Dimethylnaphthalene	$C_{12}H_{12}$	156		102 - 104	1.008	15.6 g

[a] 10 mL is not the equimolar amount for this compound; 19.5 mL is.

[b] cis- and trans-decalin were not available when this model compound was prepared.

[c] C_{20}.

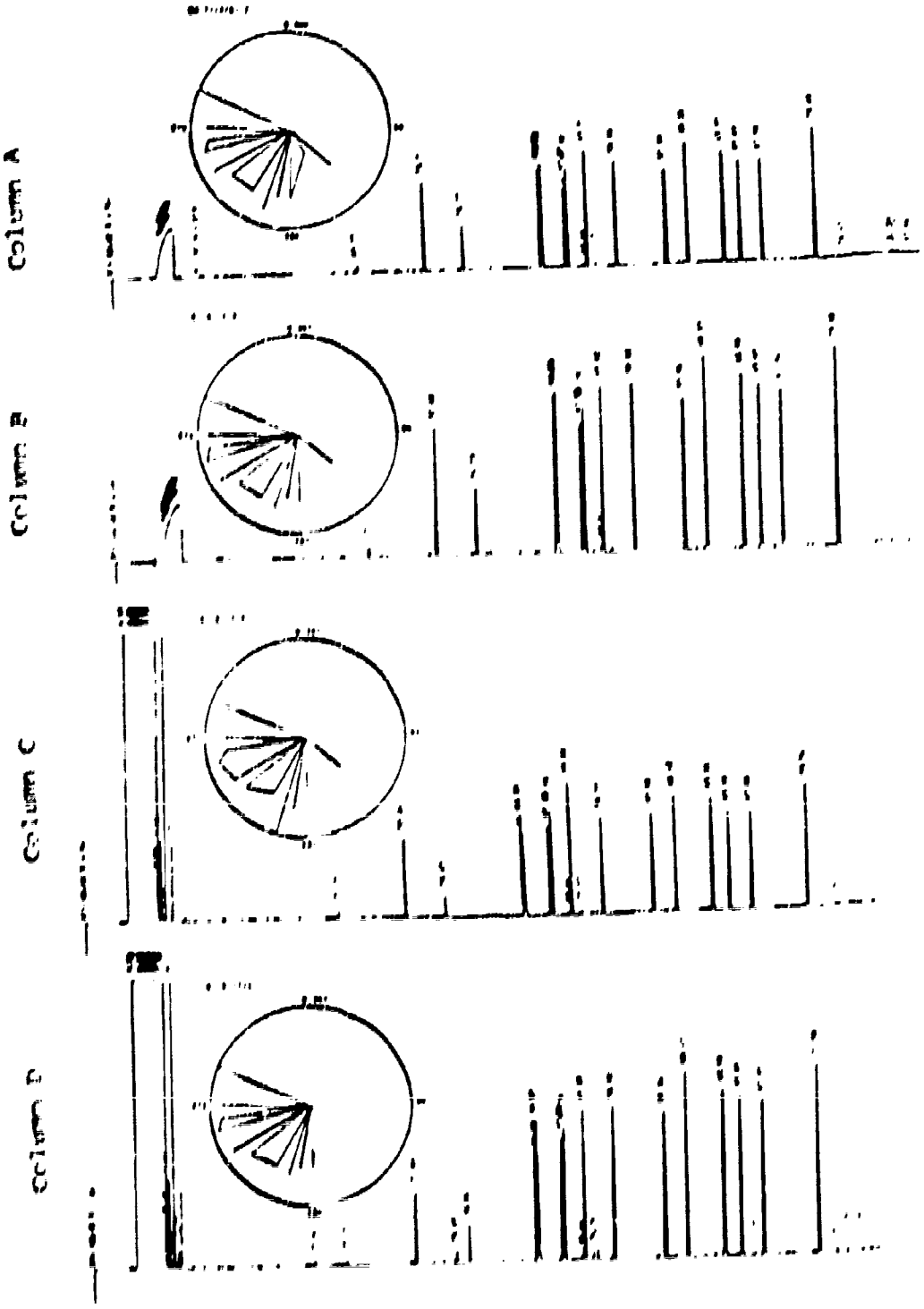

Figure B-9. Gas chromatograms of model JP-5 biodegradation plotted using computerized circular profiling - C_6 standard, day 0, 3, 6.

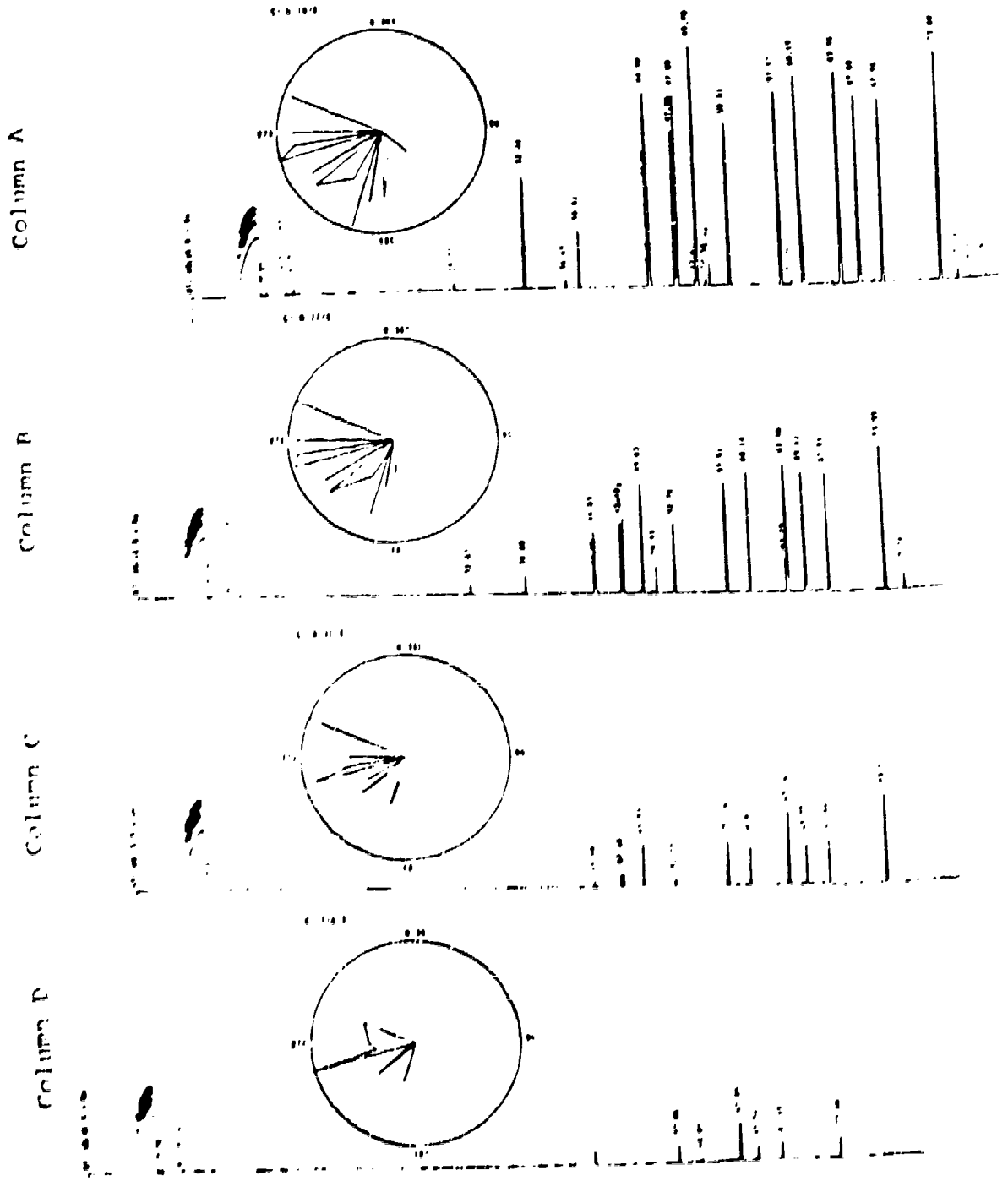

Figure B-10. Gas chromatogram of model JP-5 biodegradation plotted using computerized circular profiling, day 7, 10, 18, 24.

APPENDIX C

TRANSPORT OF SHALE-DERIVED JP-4 COMPONENTS IN LABORATORY CORE

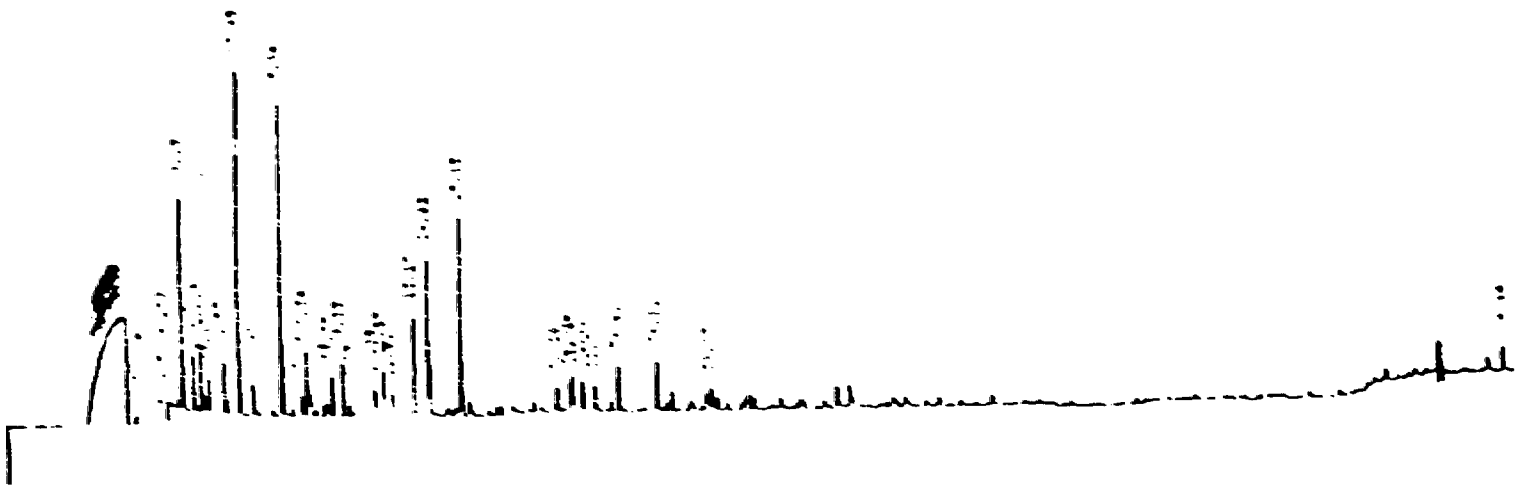

Figure C-1. Gas chromatogram of component(s) extracted from Core 10's position 1 on day 50.

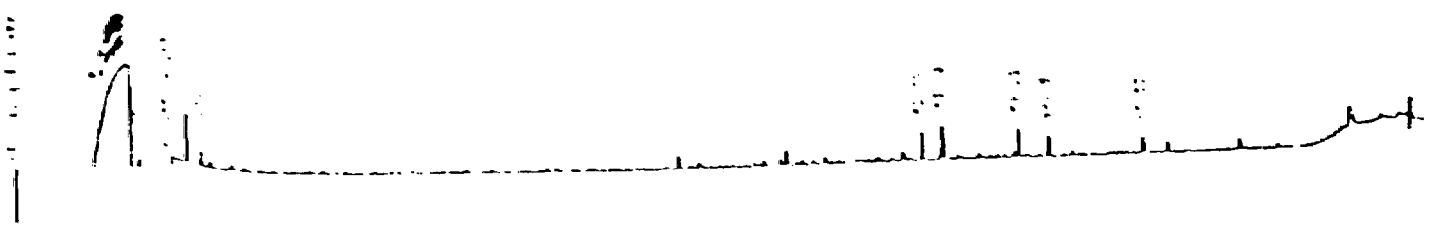

Figure C-2. Gas chromatogram of component(s) extracted from Core 10's position 1 on day 77.

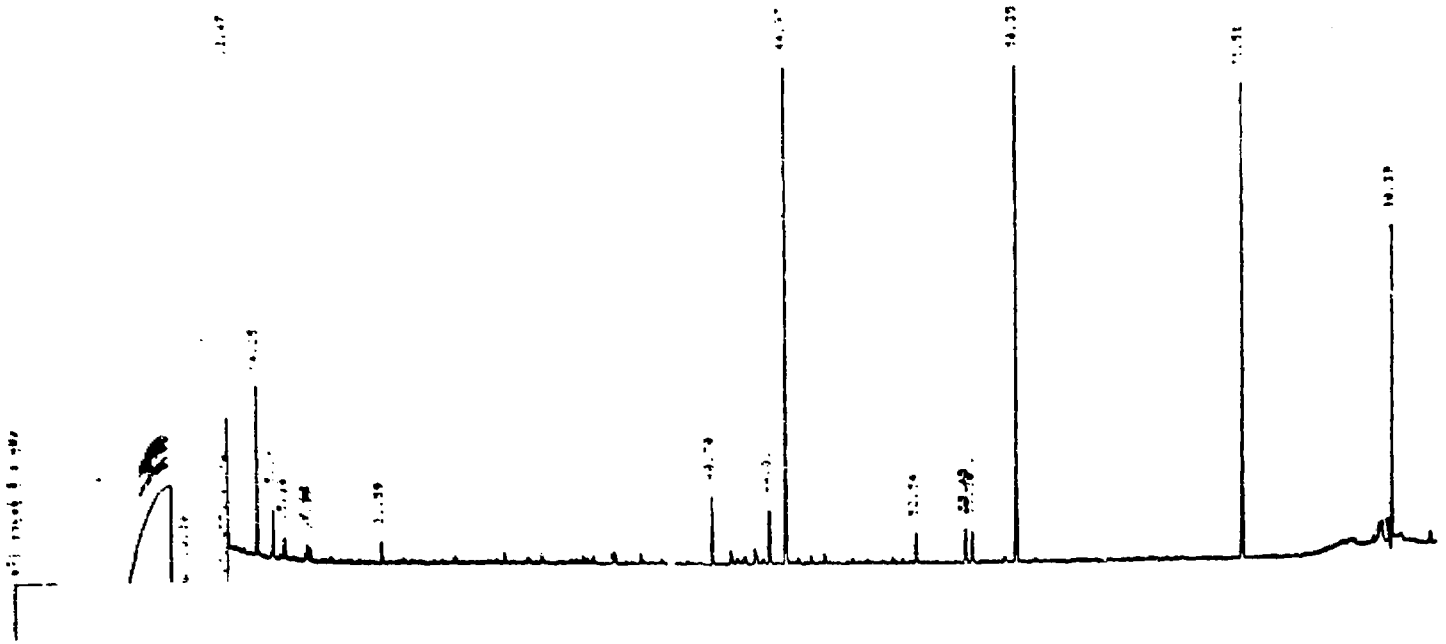

Figure C-3. Gas chromatogram of component(s) extracted from Core 10's position 1 on day 134.

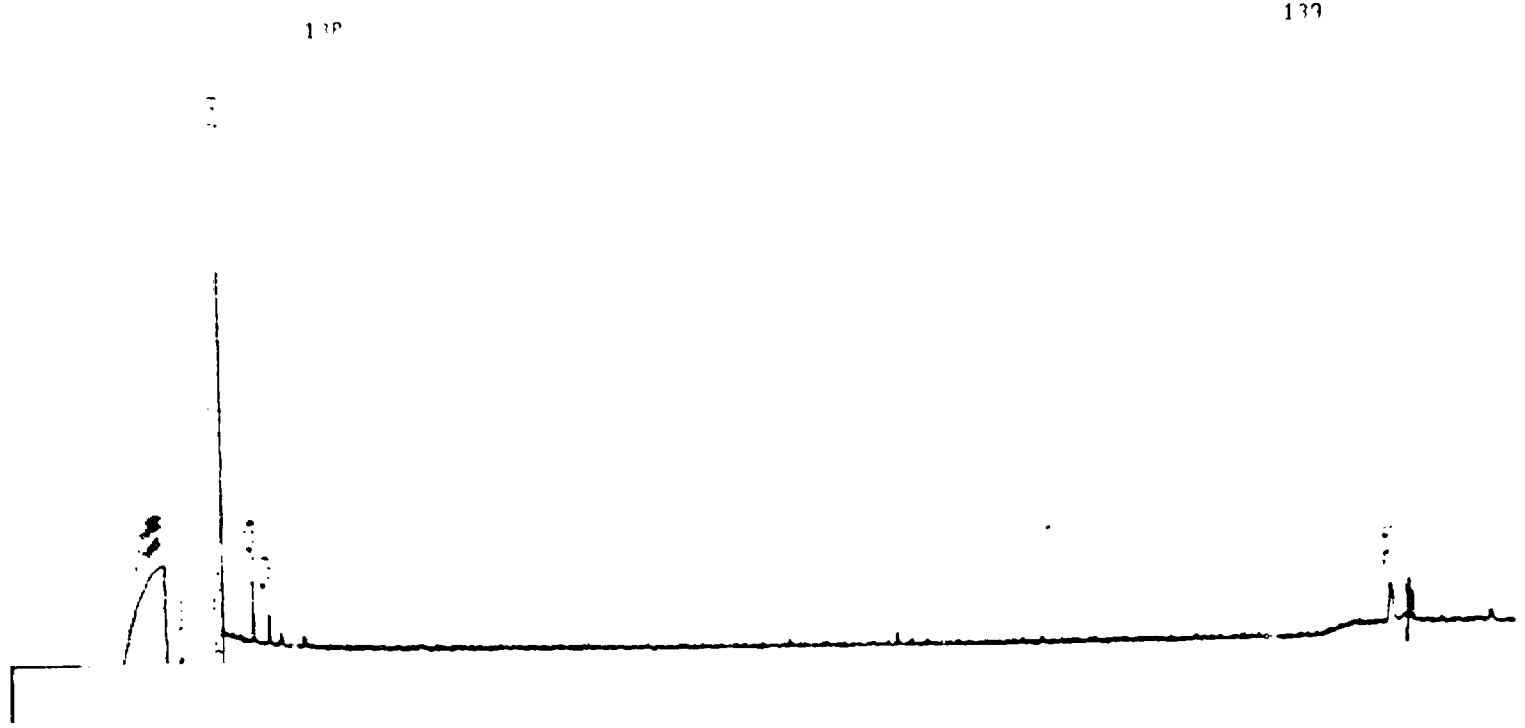

Figure C-4. Gas chromatogram of component(s) extracted from Core 10's position 2 on day 162.

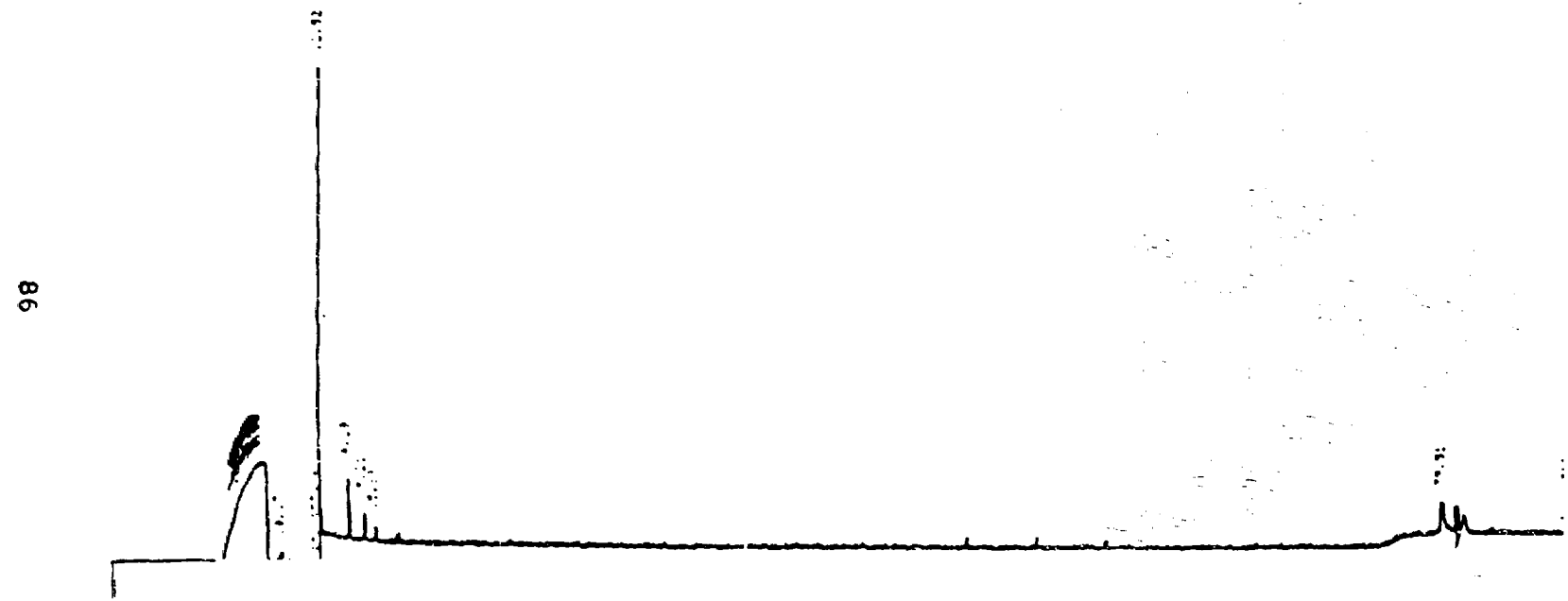

Figure C-5. Gas chromatogram of component(s) extracted from Core 10's position 2 on day 176.

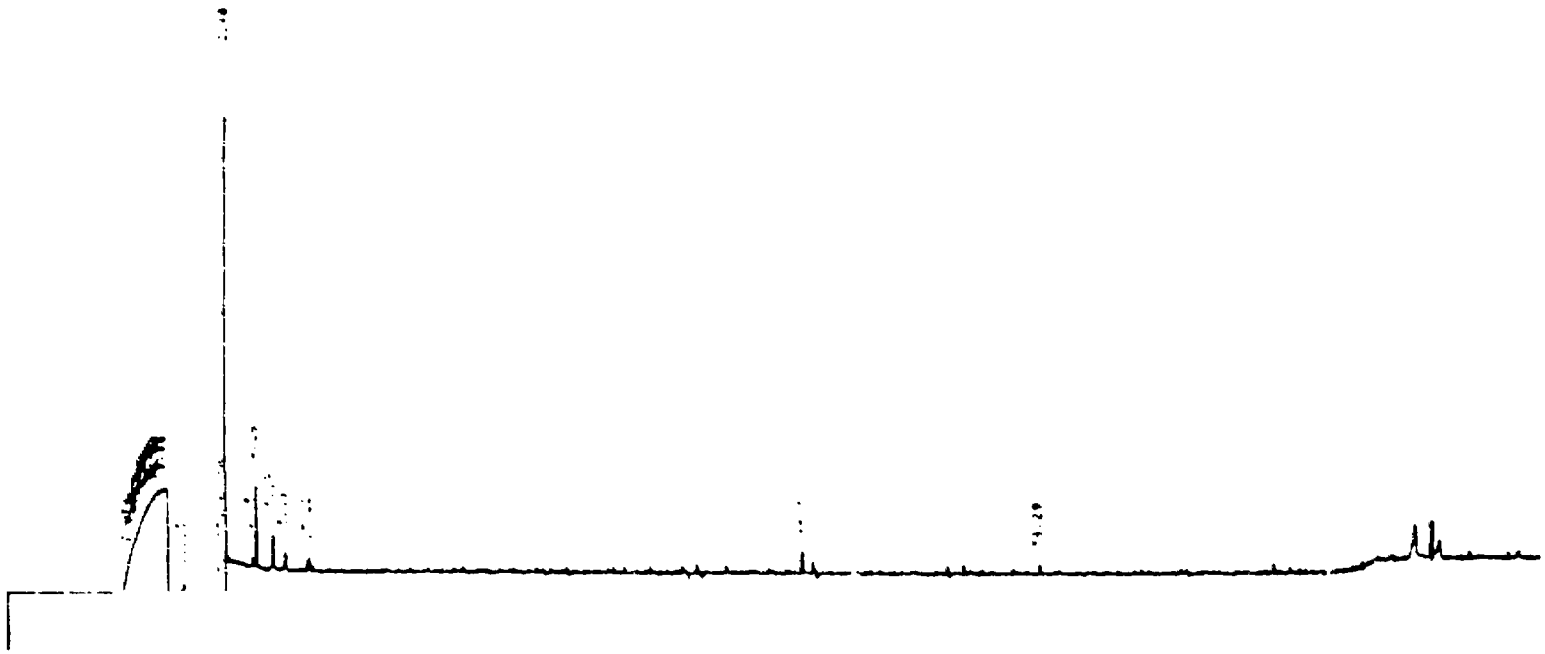

Figure C-6. Gas chromatogram of component(s) extracted from Core 10's position 4 on day 120.

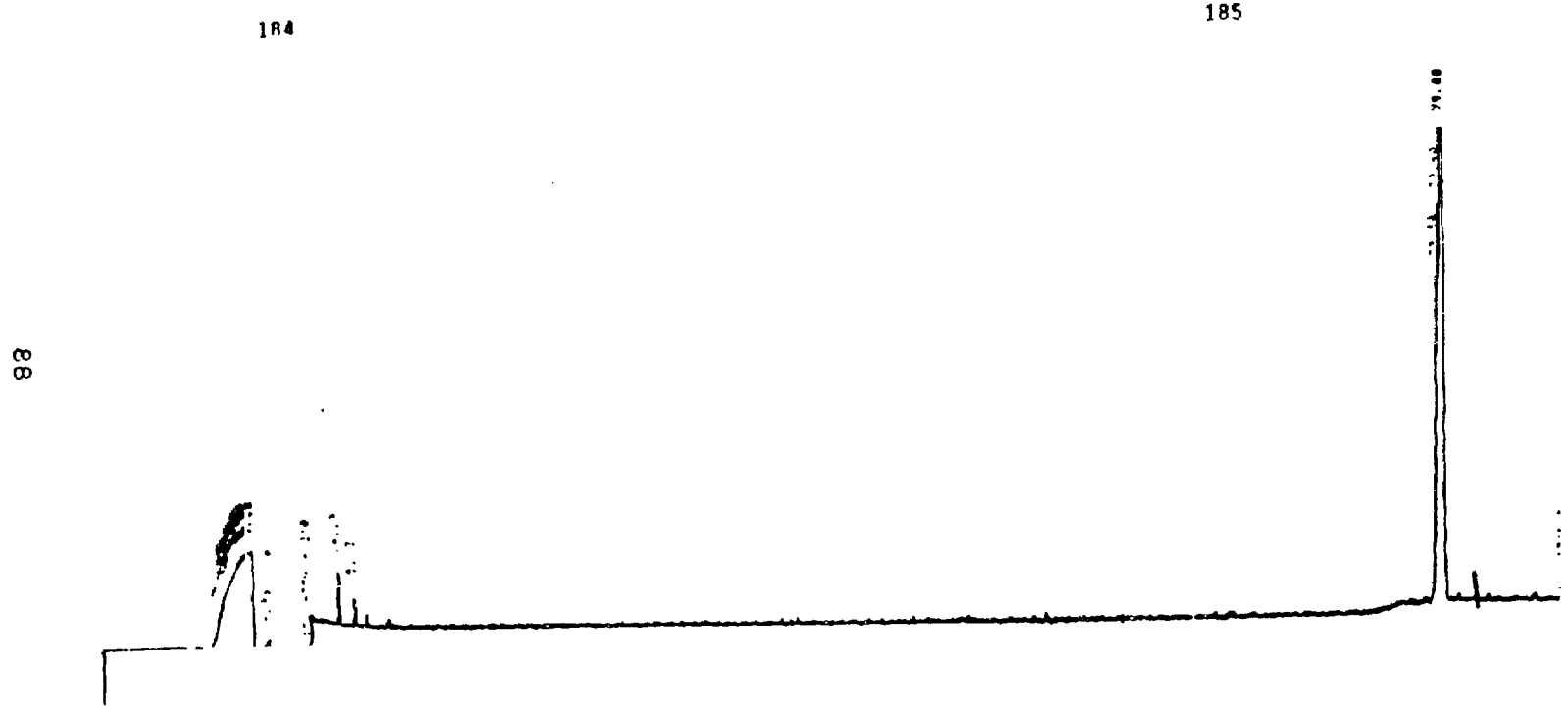

Figure C-7. Gas chromatogram of component(s) extracted from Core 10's position 4 on day 134.

Figure C-8. Gas chromatogram of component(s) extracted from Core 10's bottom position on day 197.

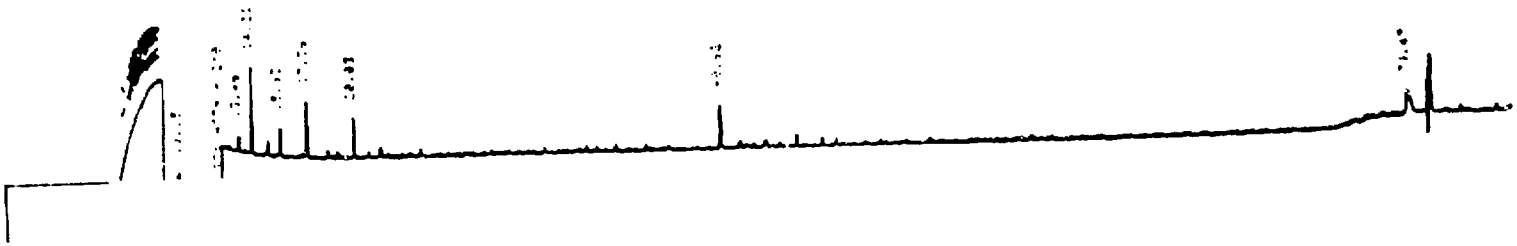

Figure C-9. Gas chromatogram of component(s) extracted from Core 10's bottom position on day 238.

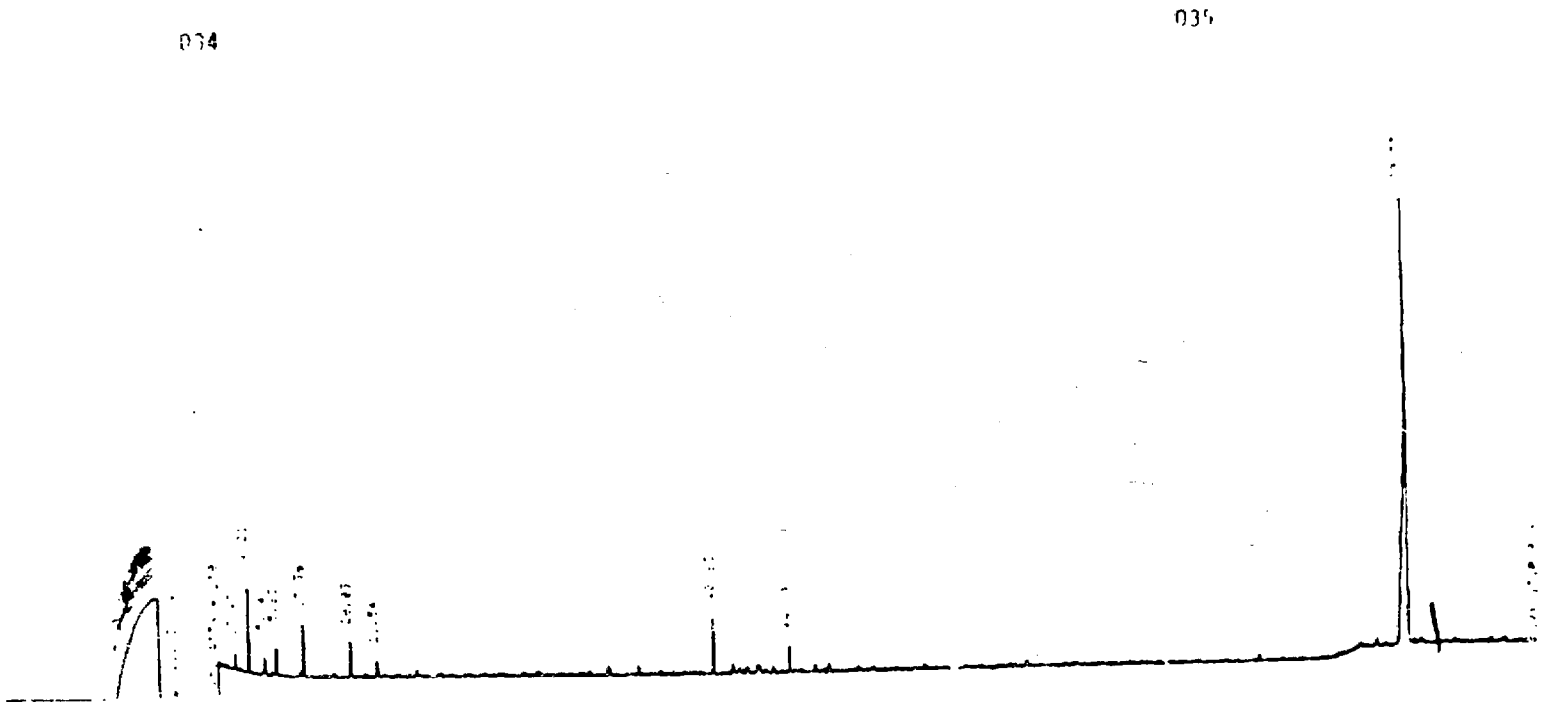

Figure C-10. Gas chromatogram of component(s) extracted from Core 10's bottom position on day 252.

TABLE C-1. TRANSPORT SUMMARY OF SHALE DERIVED JP-4 THROUGH LABORATORY CORE 10[a]

Probe position	Day															
	50	64	77	92	106	120	134	148	162	176	184	197	211	224	238	252
1	2	0	2	0	0	0	3	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
3	0	0	0	0	0	0	0	-	0	-	-	0	0	0	0	0
4	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
Bottom	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	2

[a]Values reported represent the number of peaks identified by GC analysis. Dashes indicate that no GC analysis was run on that day.

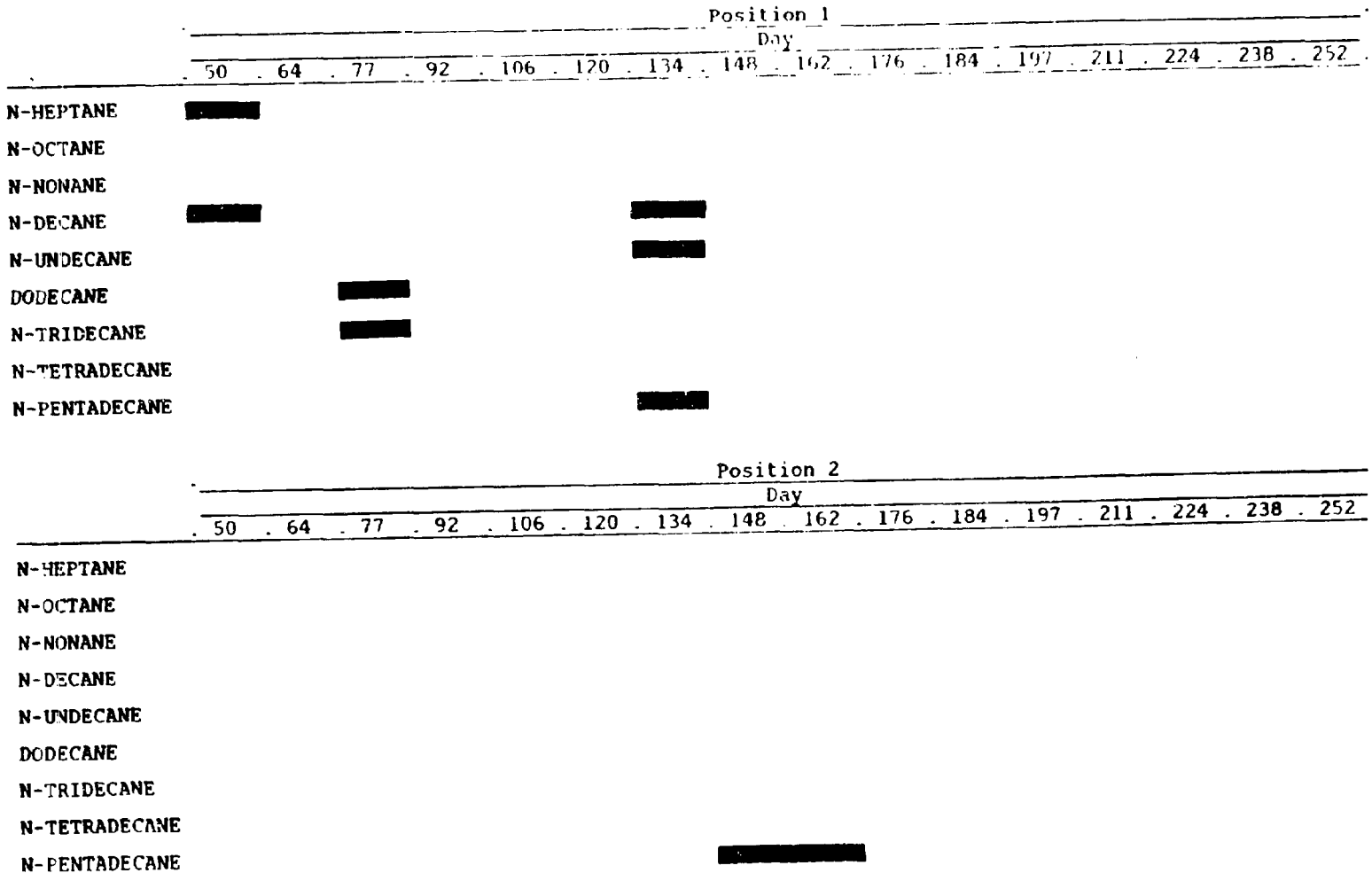

Figure C-11. Transport of selected components in shale derived JP-4 through Core 10.

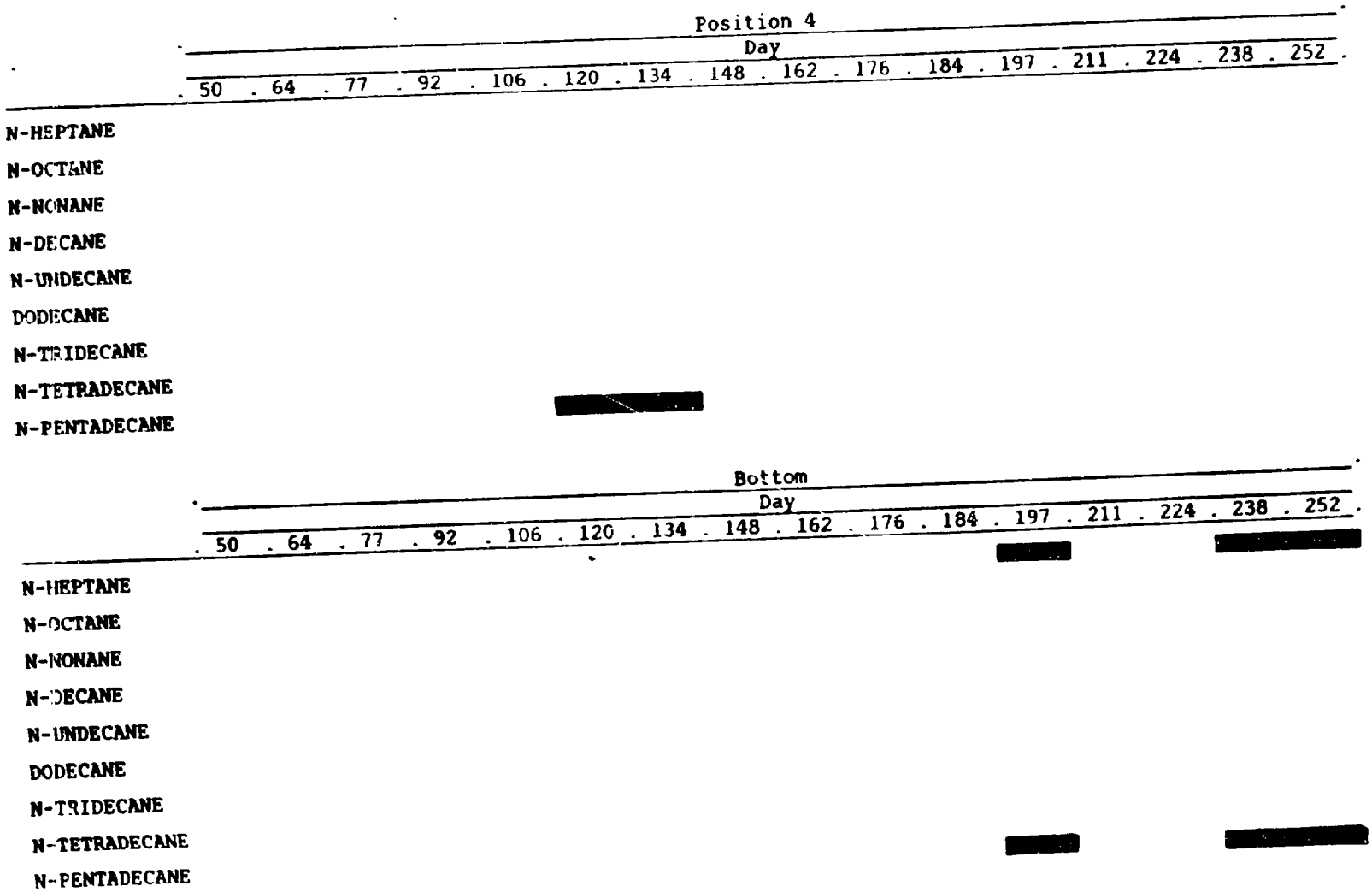

Figure C-11 (continued)

APPENDIX D

TRANSPORT OF MODEL JP-5 IN LABORATORY CORE

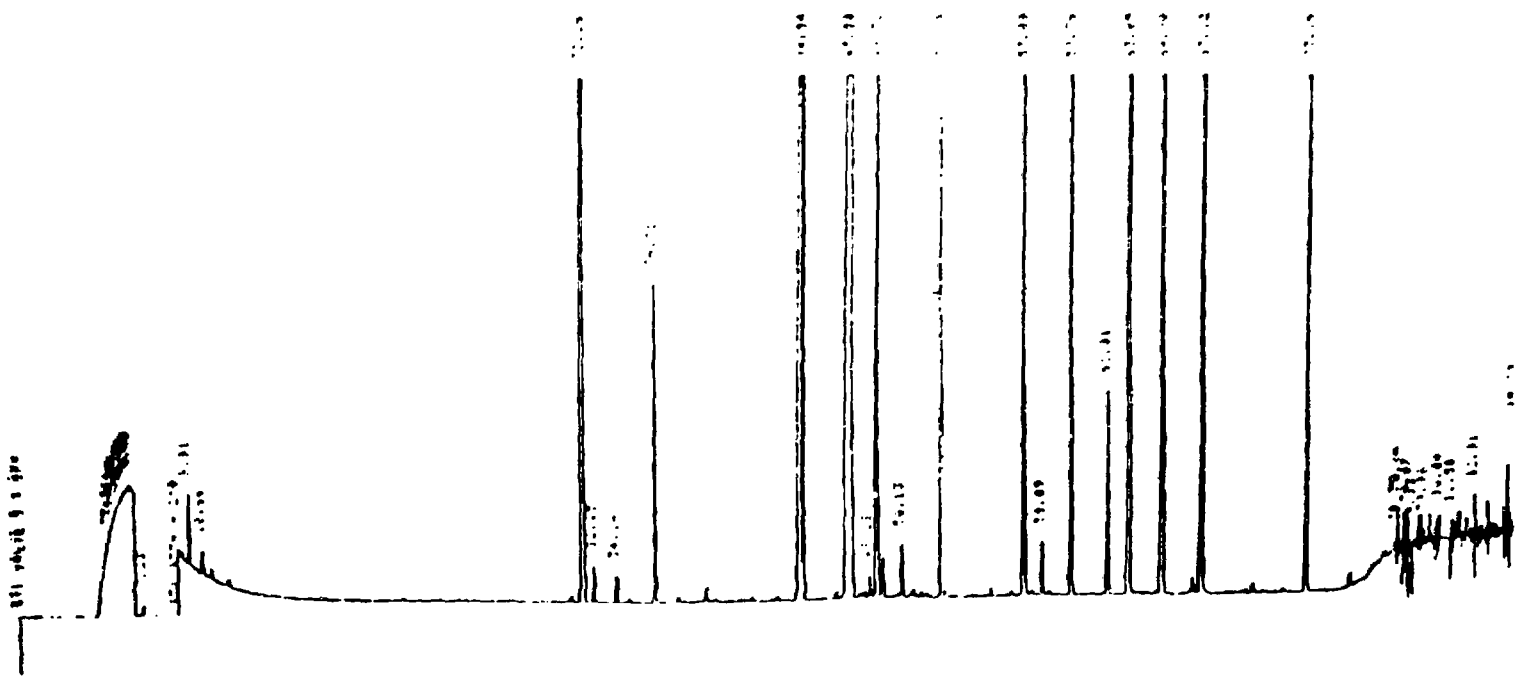

Figure D-1. Gas chromatogram of component(s) extracted from Core 12, position 1 on day 54.

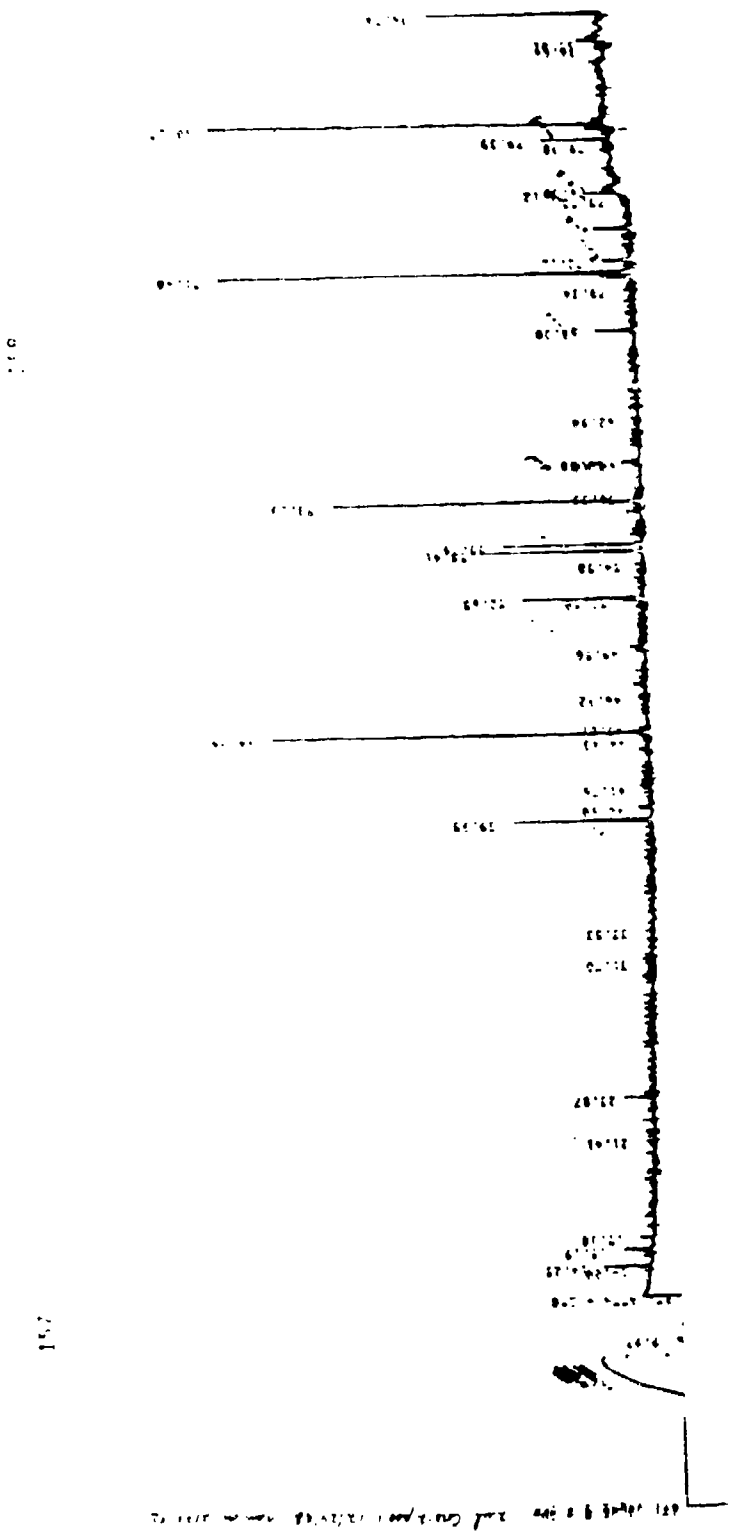

Figure D-2. Gas chromatogram of component(s) extracted from Core 12, position 1 on day 62.

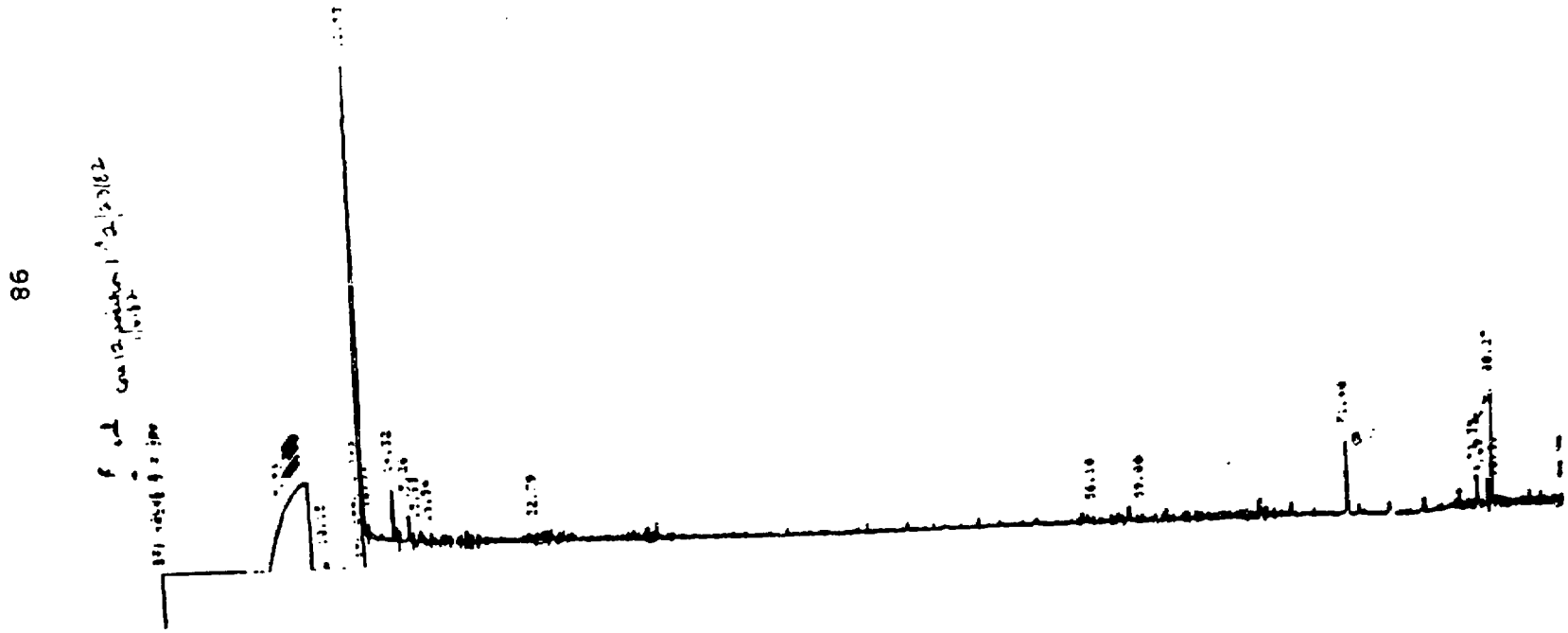

Figure D-3. Gas chromatogram of component(s) extracted from Core 12, position 1 on day 75.

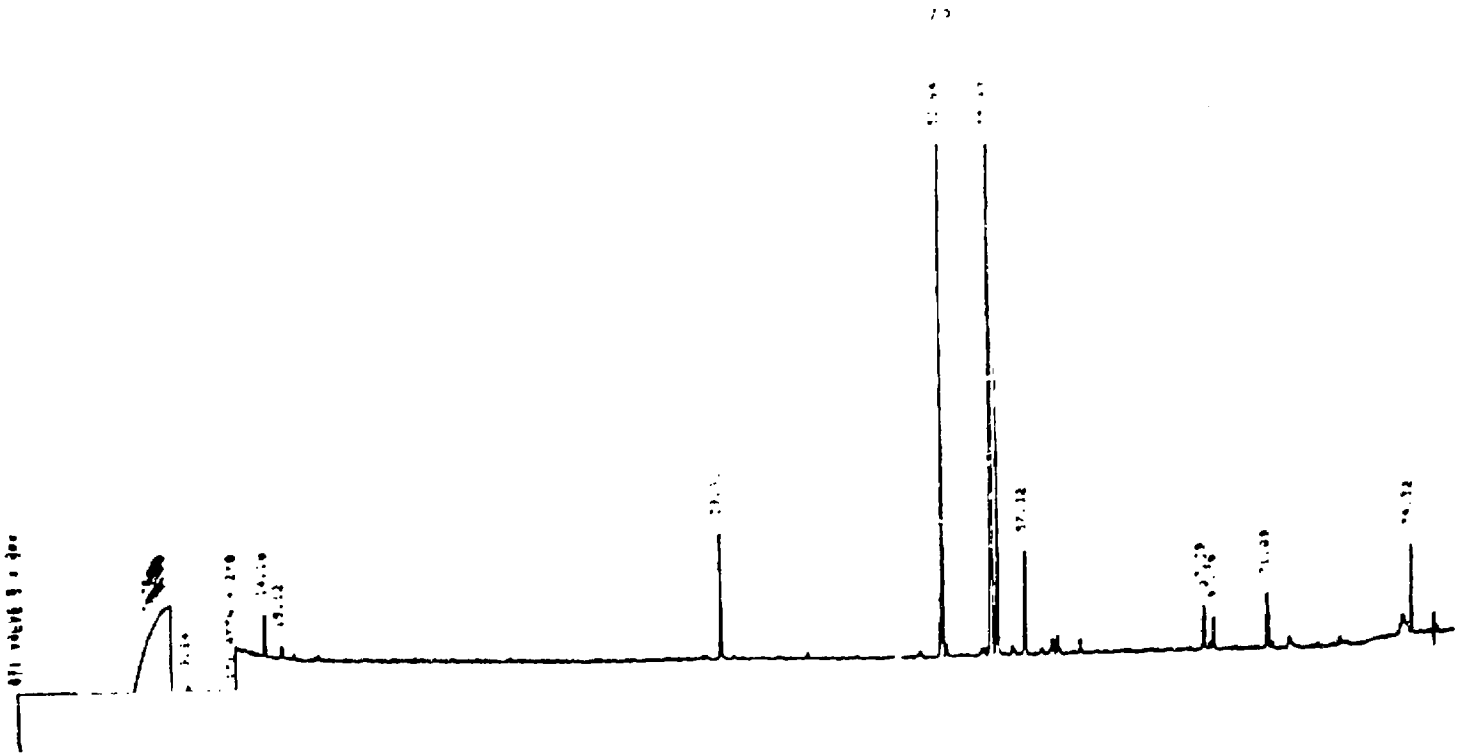

Figure D-4. Gas chromatogram of component(s) extracted from Core 12, position 1 on day 89.

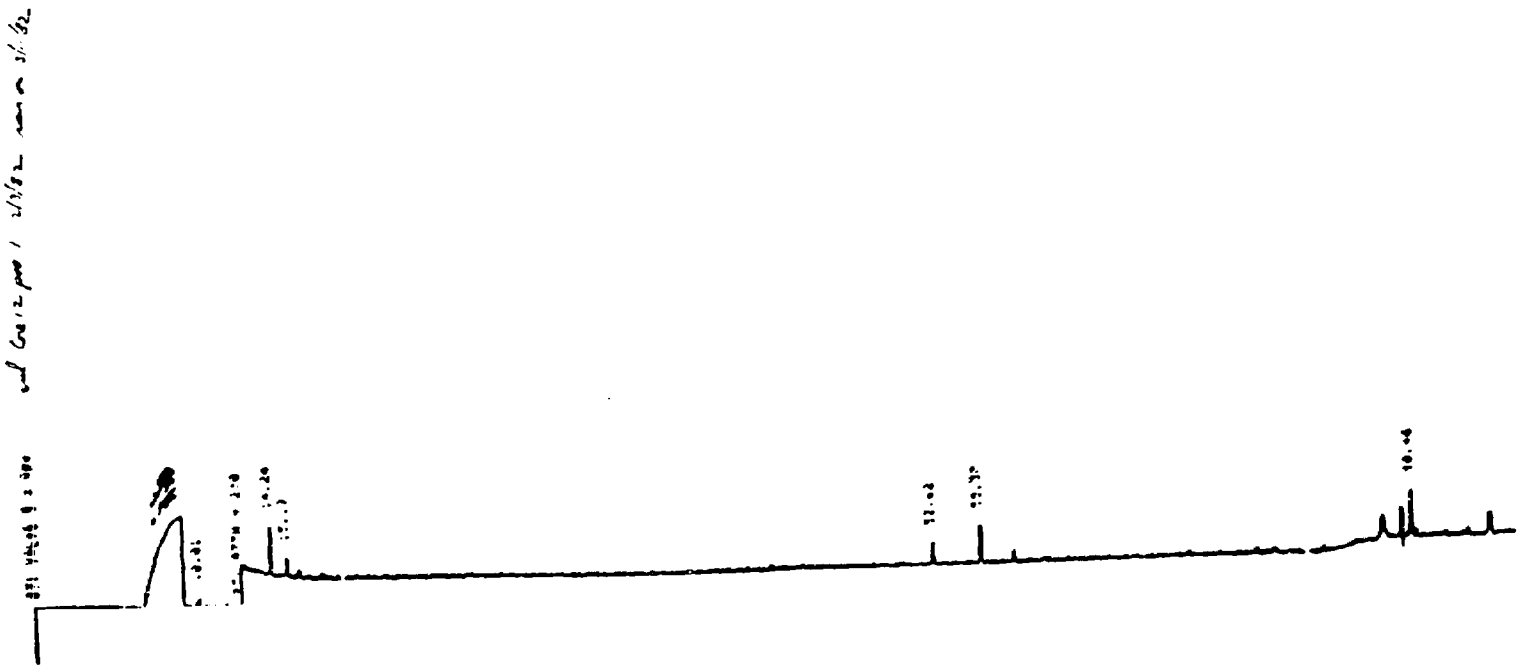

Figure D-5. Gas chromatogram of component(s) extracted from Core 12, position 1 on day 103.

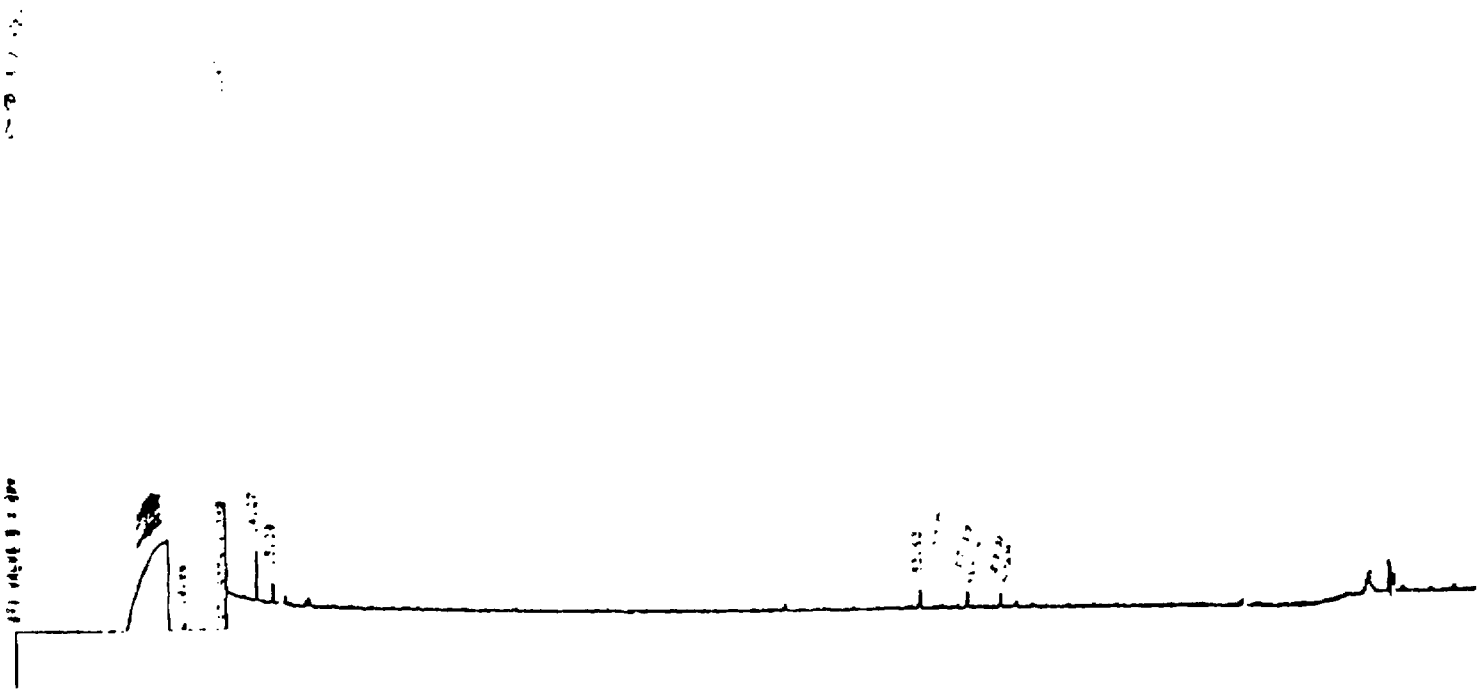

Figure D-6. Gas chromatogram of component(s) extracted from Core 12, position 1 on day 117.

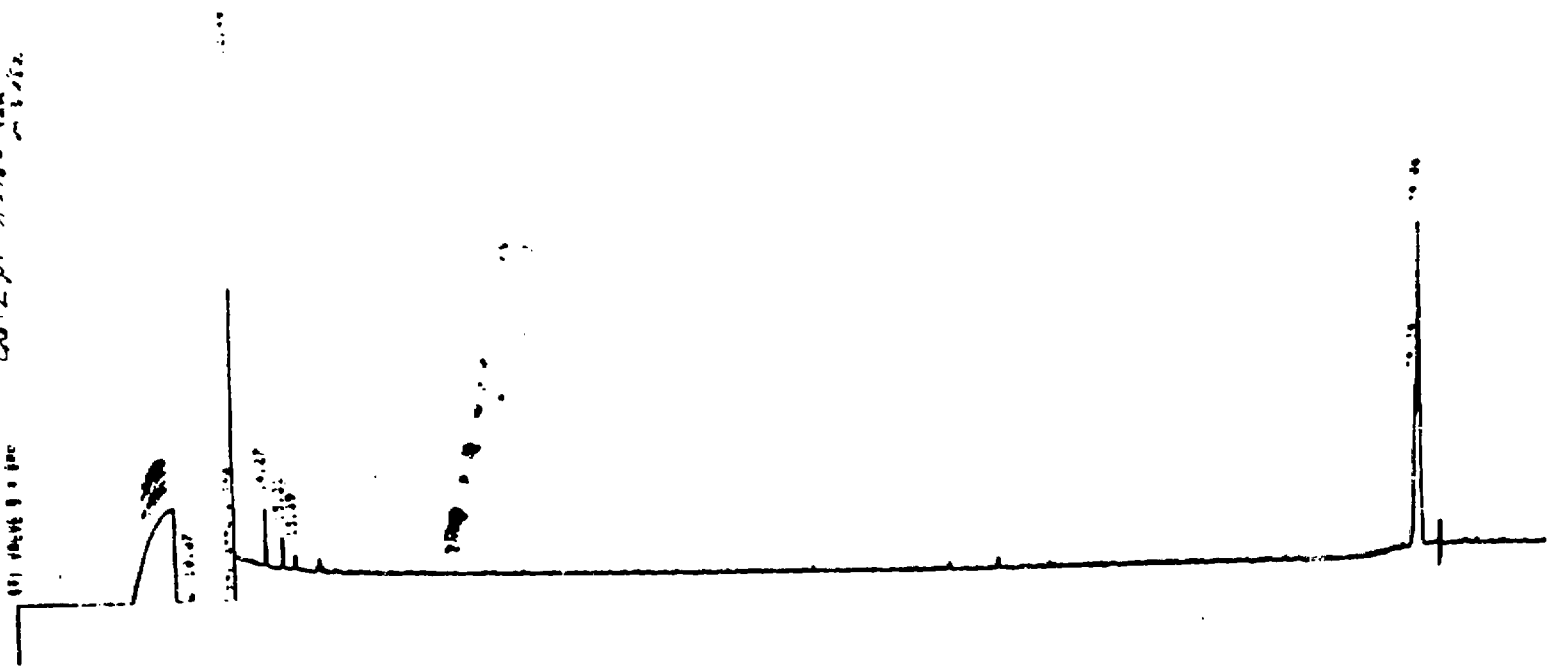

Figure D-7. Gas chromatogram of component(s) extracted from Core 12, position 1 on day 131.

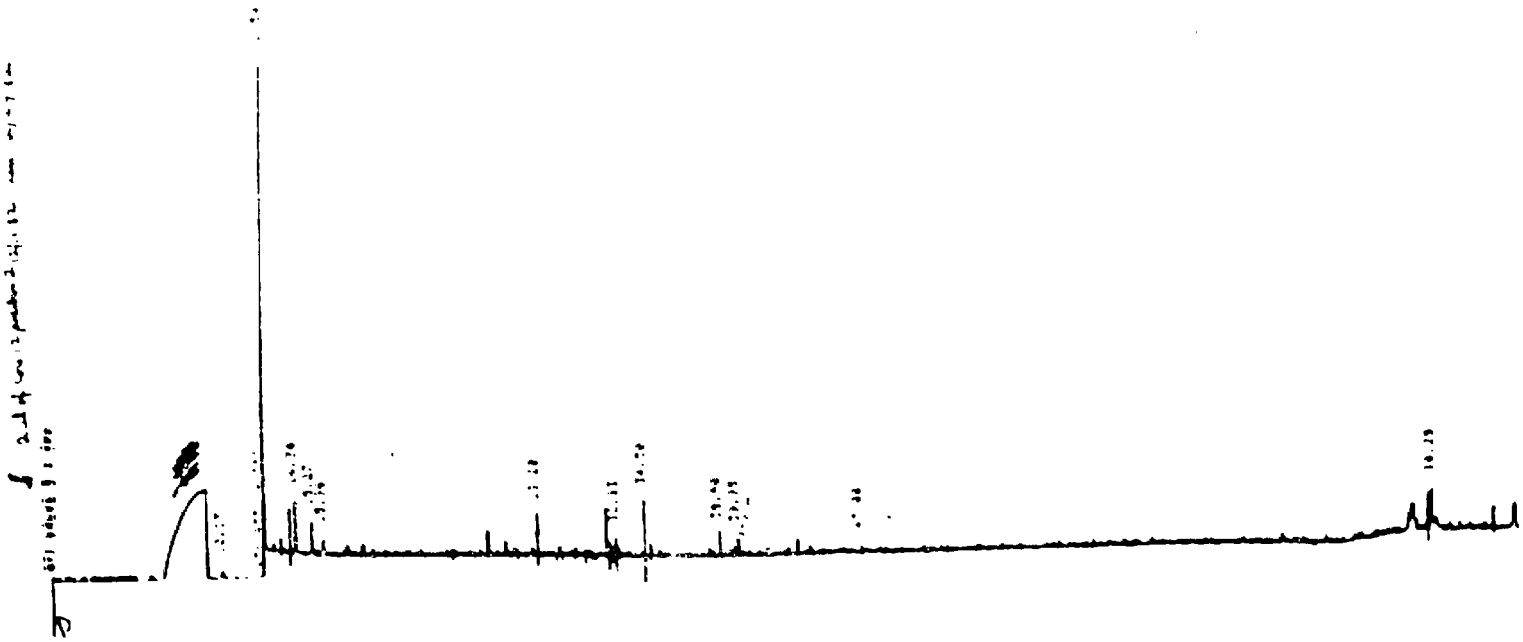

Figure D-8. Gas chromatogram of component(s) extracted from Core 12, position 2 on day 54.

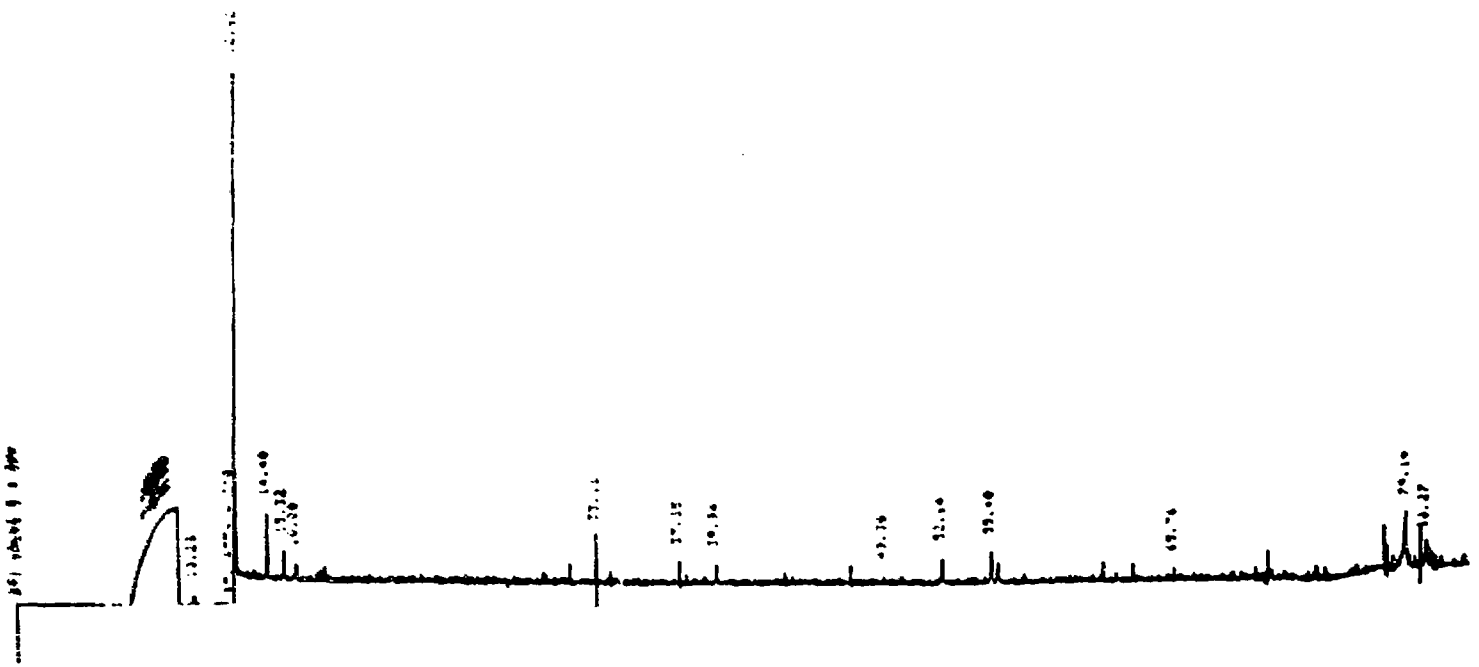

Figure D-9. Gas chromatogram of component(s) extracted from Core 12, position 2 on day 62.

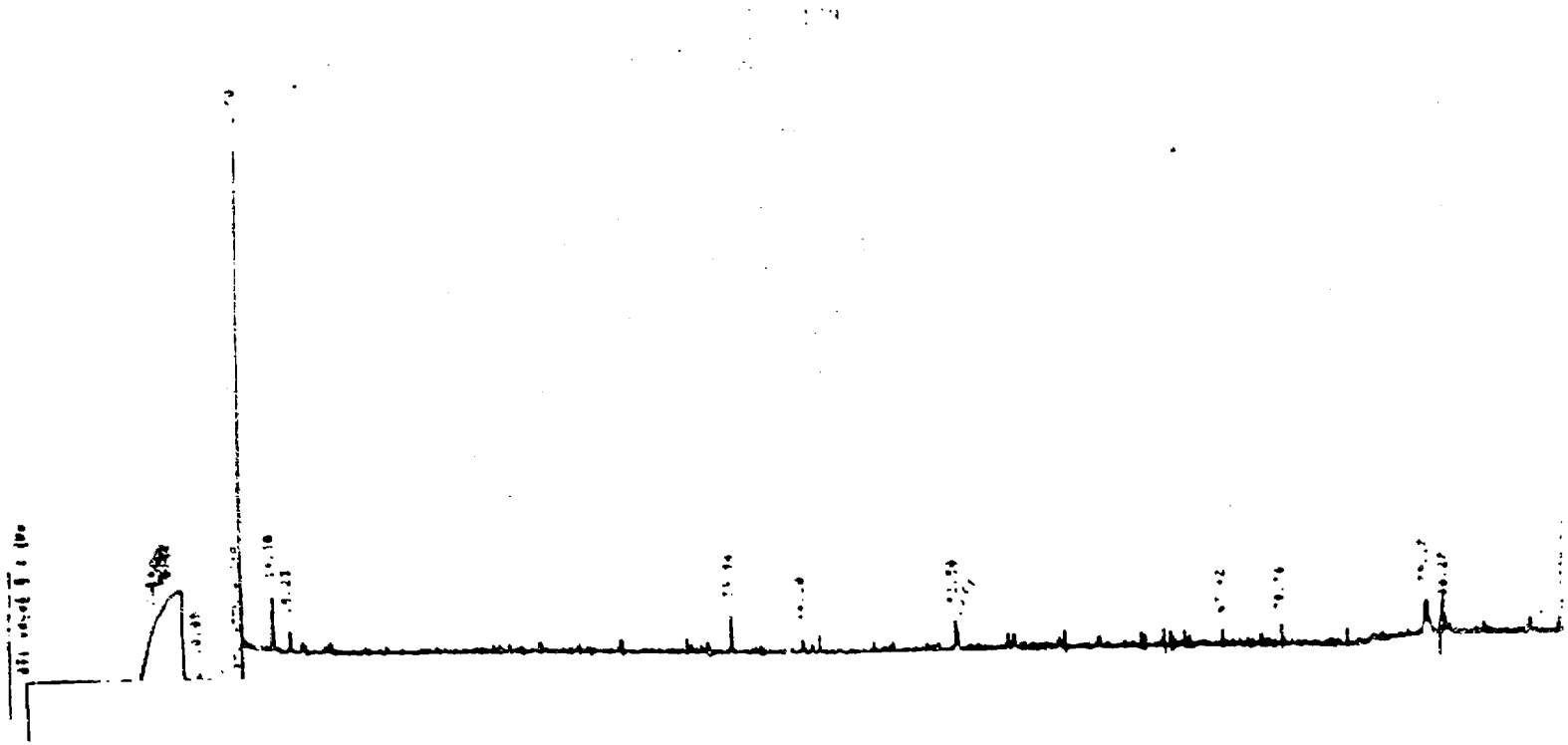

Figure D-10. Gas chromatogram of component(s) extracted from Core 12, position 2 on day 89.

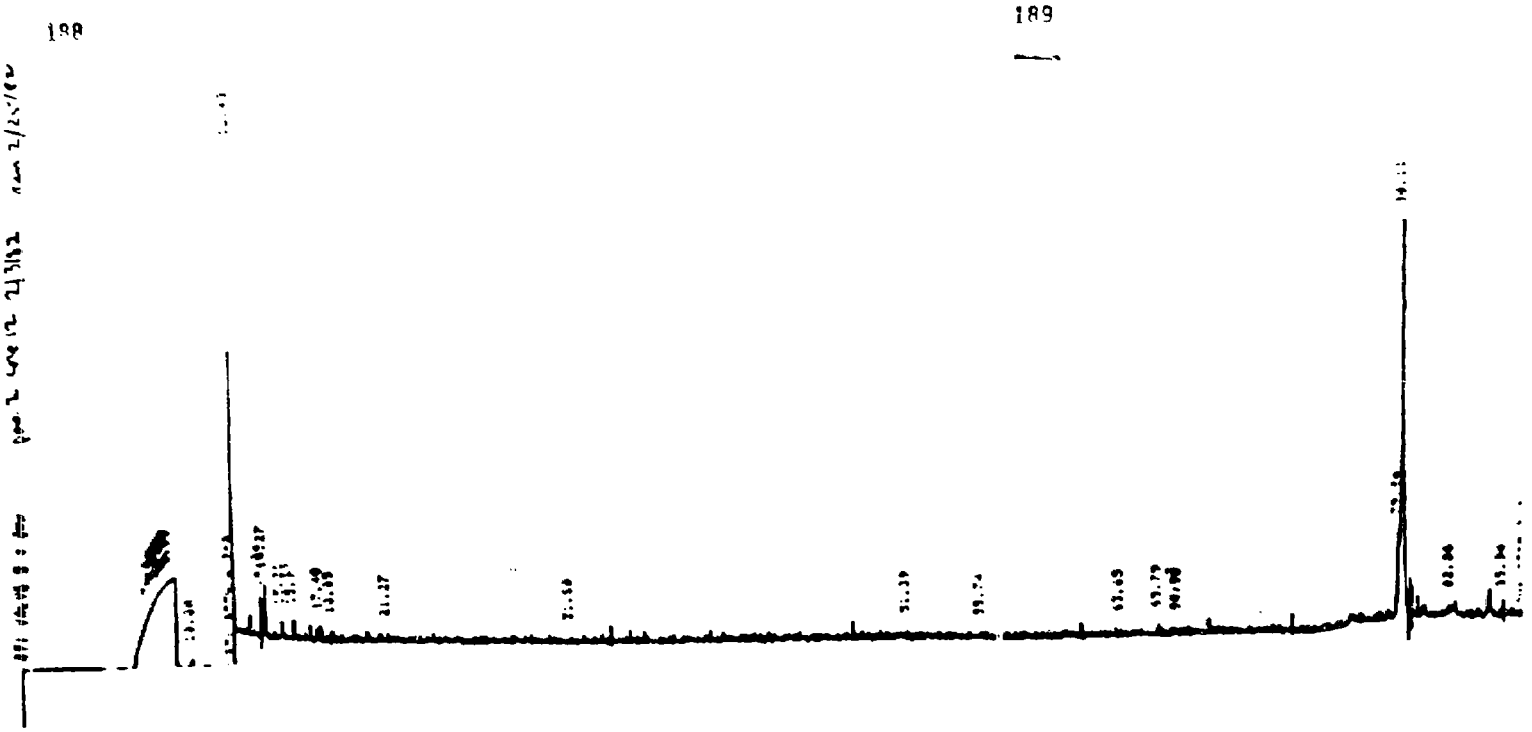

Figure D-11. Gas chromatogram of component(s) extracted from Core 12, position 2 on day 103.

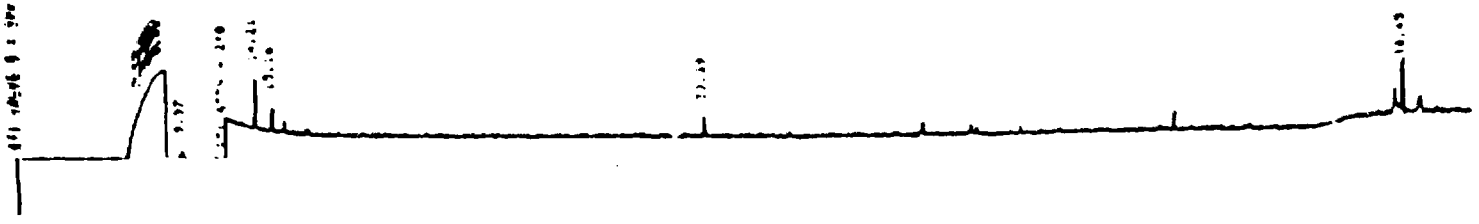

Figure D-12. Gas chromatogram of component(s) extracted from Core 12, position 5 on day 75.

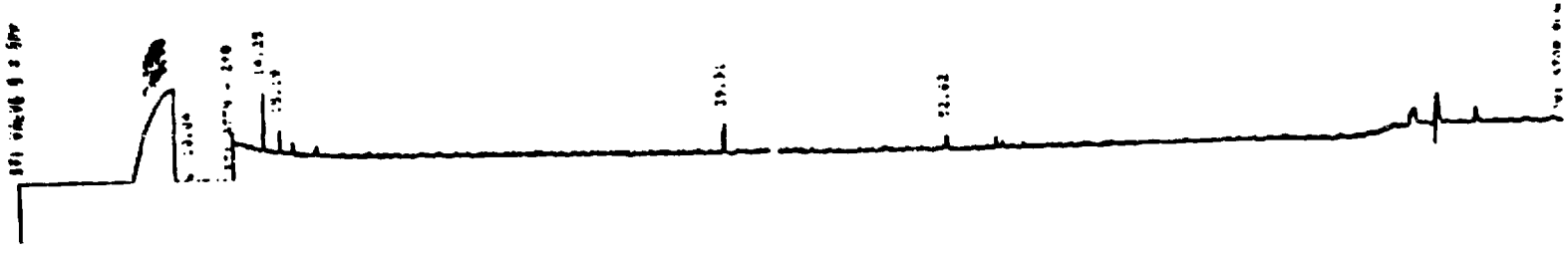

Figure D-13. Gas chromatogram of component(s) extracted from Core 12, position 5 on day 131.

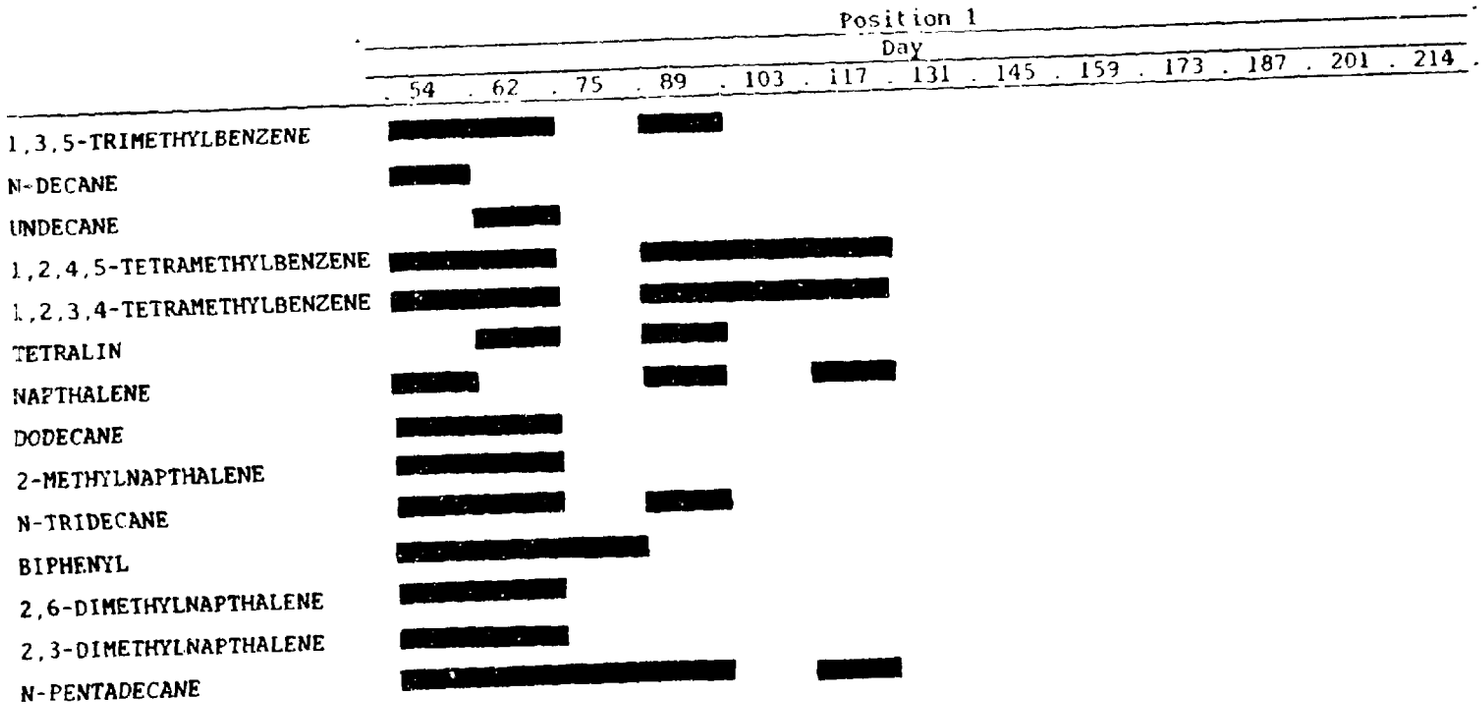

Figure D-14. Transport of Model JP-5 components in Core 12* over time.

*Core 12 was studied in indoor laboratory.

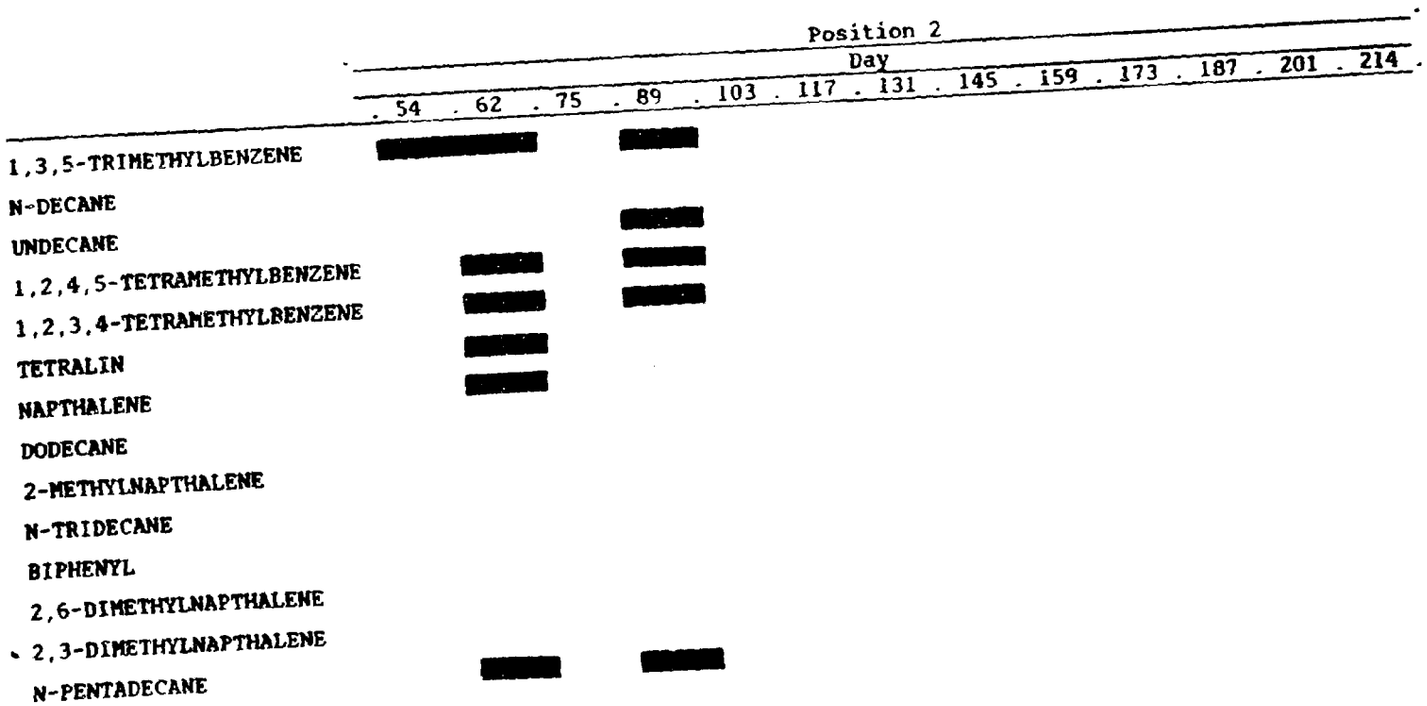

Figure D-14 (continued)

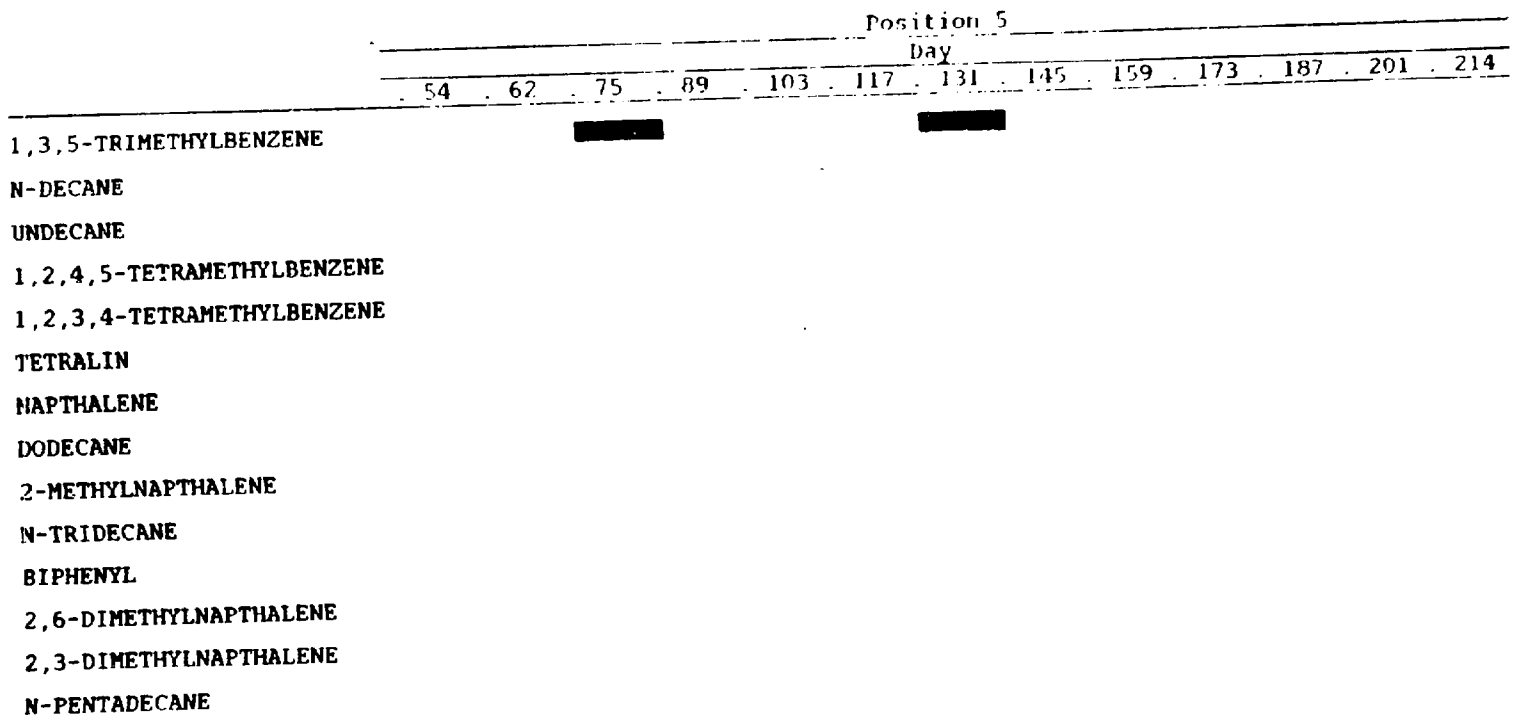

Figure D-14 (continued)

TABLE D-1. TRANSPORT SUMMARY OF MODEL JP-5 THROUGH LABORATORY CORE 12[a]

Probe position	Day												
	54	62	75	89	103	117	131	145	159	173	187	201	214
1	12	12	1	7	2	4	0	0	0	0	0	-	-
2	1	5	0	4	0	0	0	0	0	0	0	-	-
3	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	1	0	0	0	1	0	0	0	0	0	0
Bottom	0	0	0	0	0	0	0	0	0	0	0	0	0

[a]Values reported represent the number of peaks identified by GC analysis. Dashes indicate that no GC analysis was run on that day.

APPENDIX E

TRANSPORT OF MODEL JP-5 IN OUTDOOR CORE

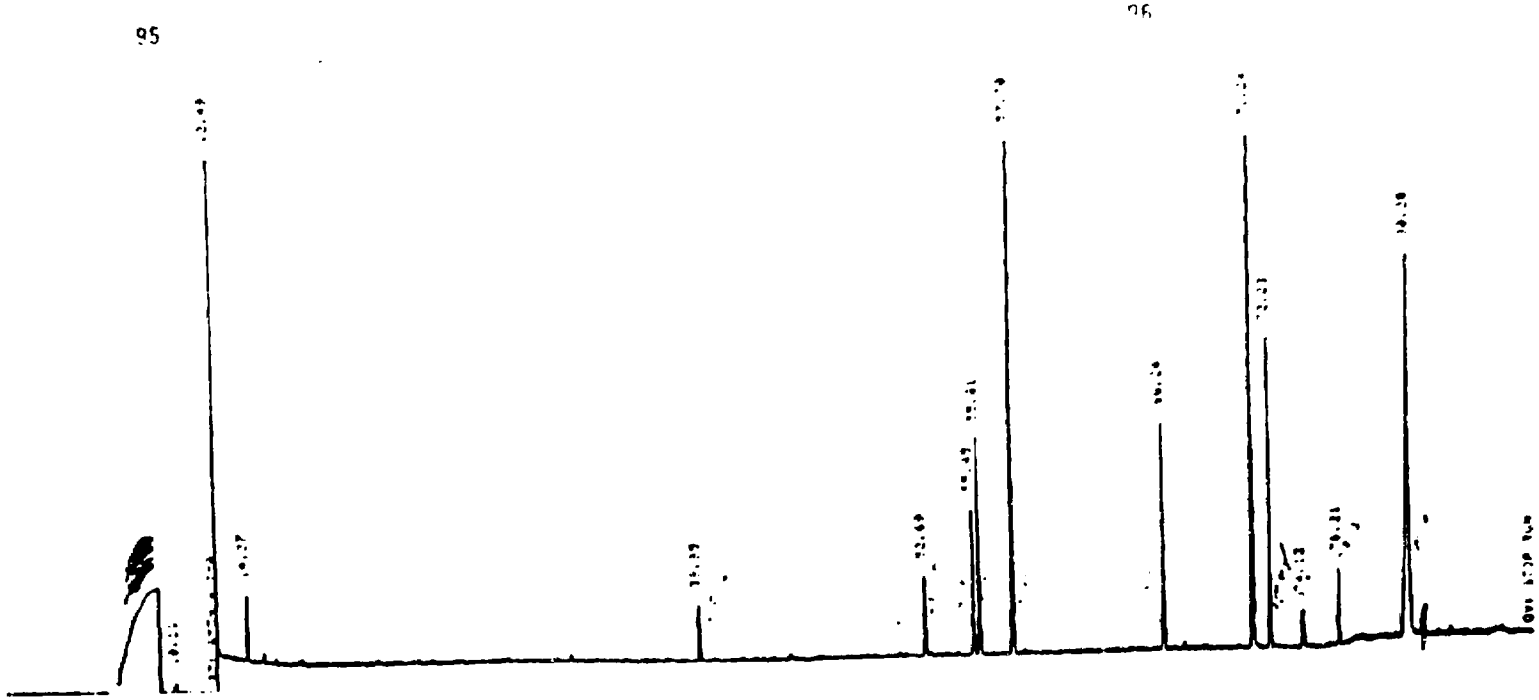

Figure E-1. Gas chromatogram of component(s) extracted from Core 13, position 1 on day 62.

Figure E-2. Gas chromatogram of component(s) extracted from Core 13, position 1 on day 75.

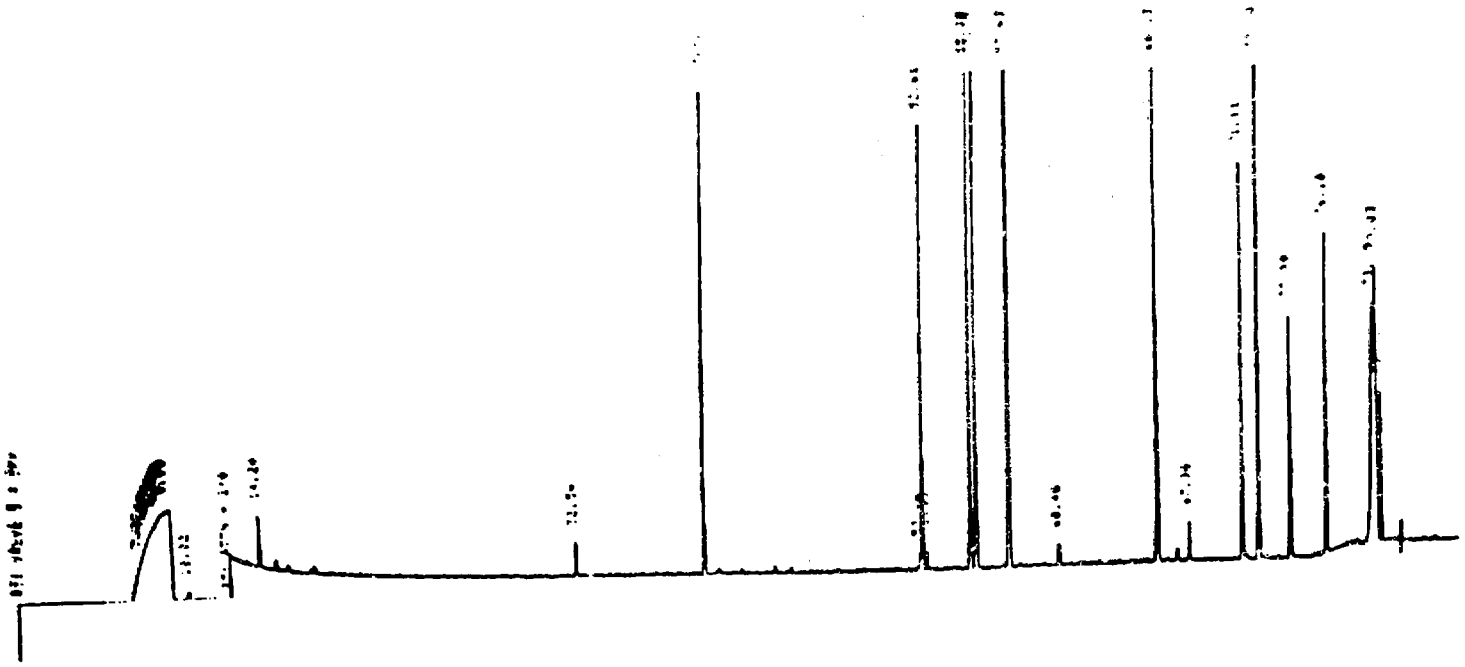

Figure E-3. Gas chromatogram of component(s) extracted from Core 13, position 1 on day 103.

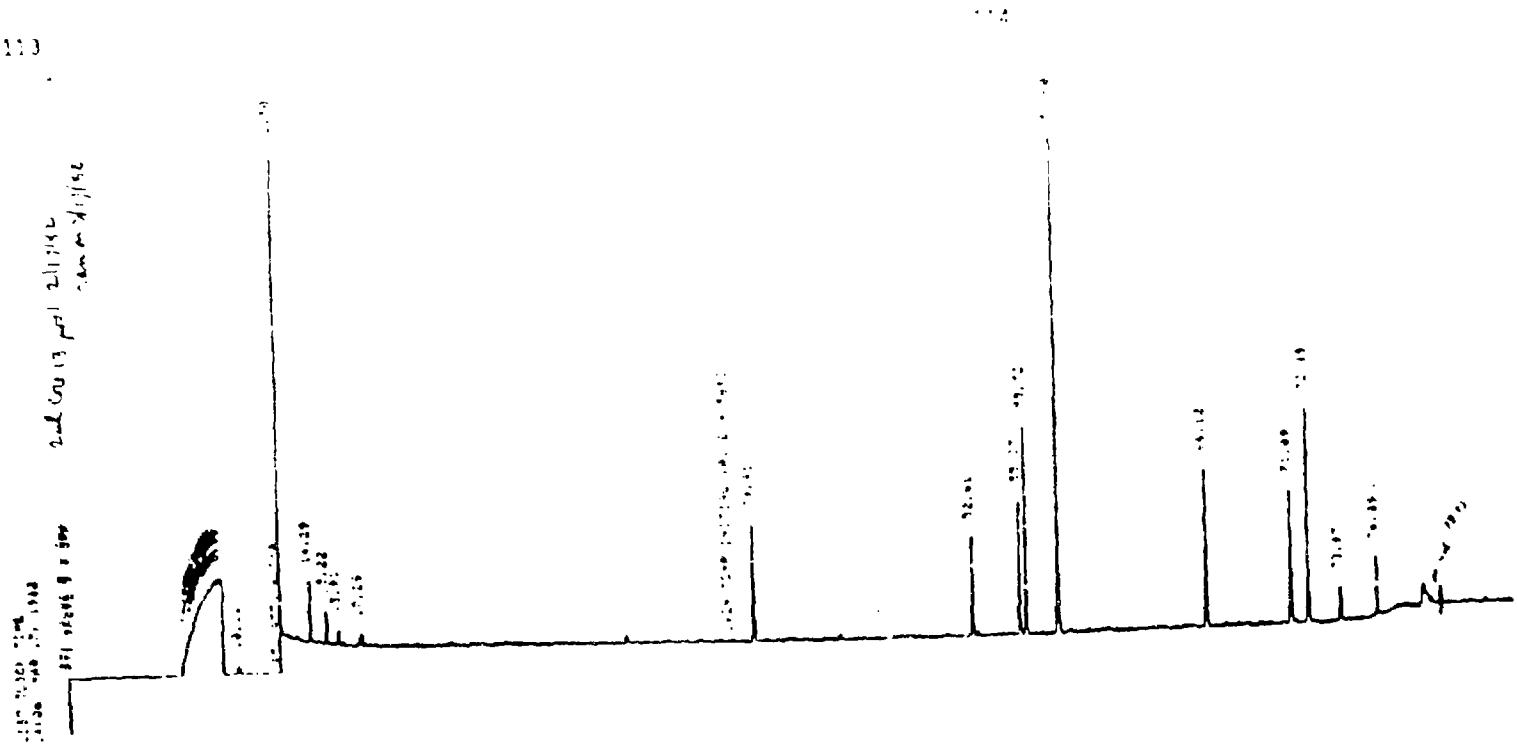

Figure E-4. Gas chromatogram of component(s) extracted from Core 13, position 1 on day 117.

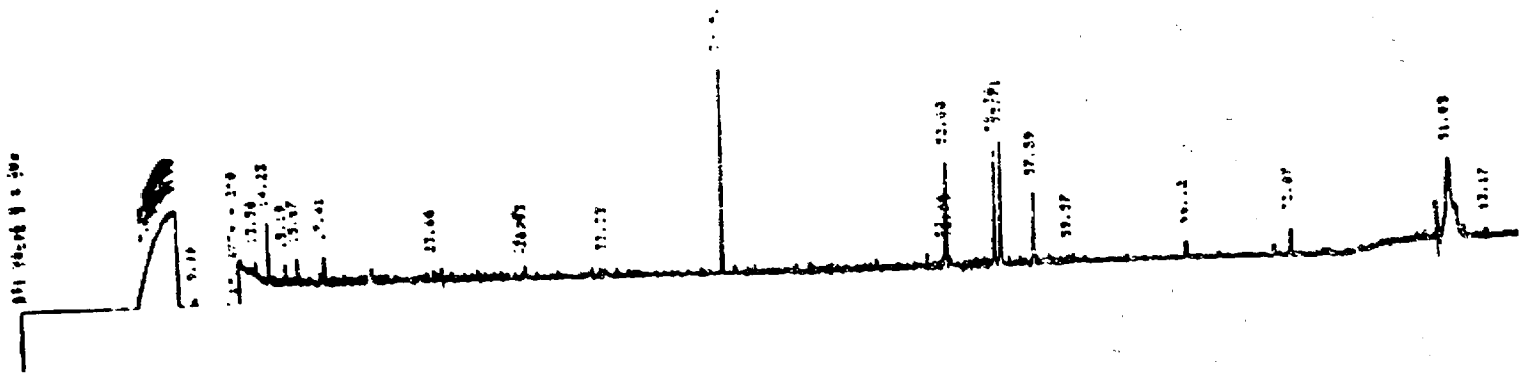

Figure E-5. Gas chromatogram of component(s) extracted from Core 13, position 1 on day 131.

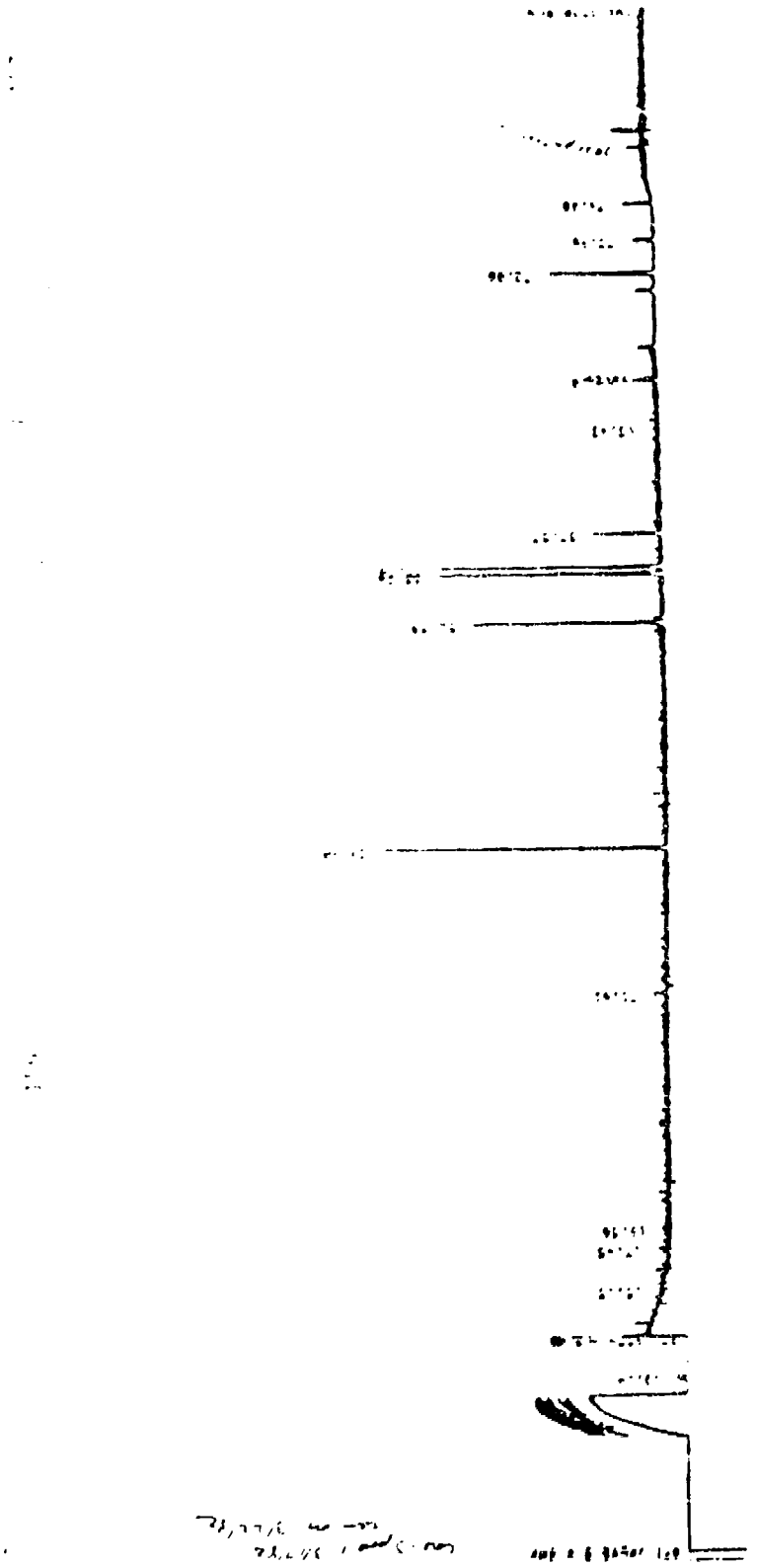

Figure E-6. Gas chromatogram of component(s) extracted from Core 13, position 1 on day 145.

Figure E-7. Gas chromatogram of component(s) extracted from Core 13, position 1 on day 159.

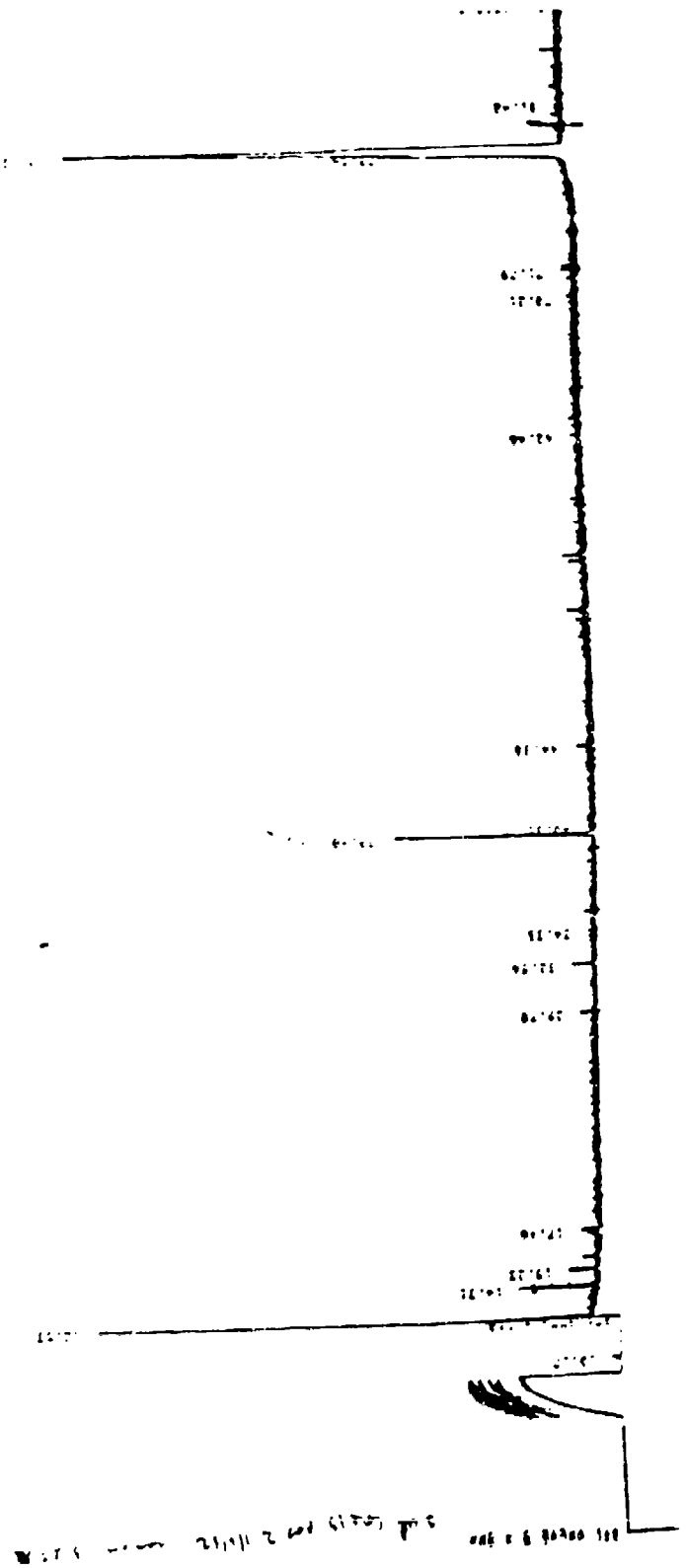

Figure E-8. Gas chromatogram of component(s) extracted from Core 13, position 2 on day 75.

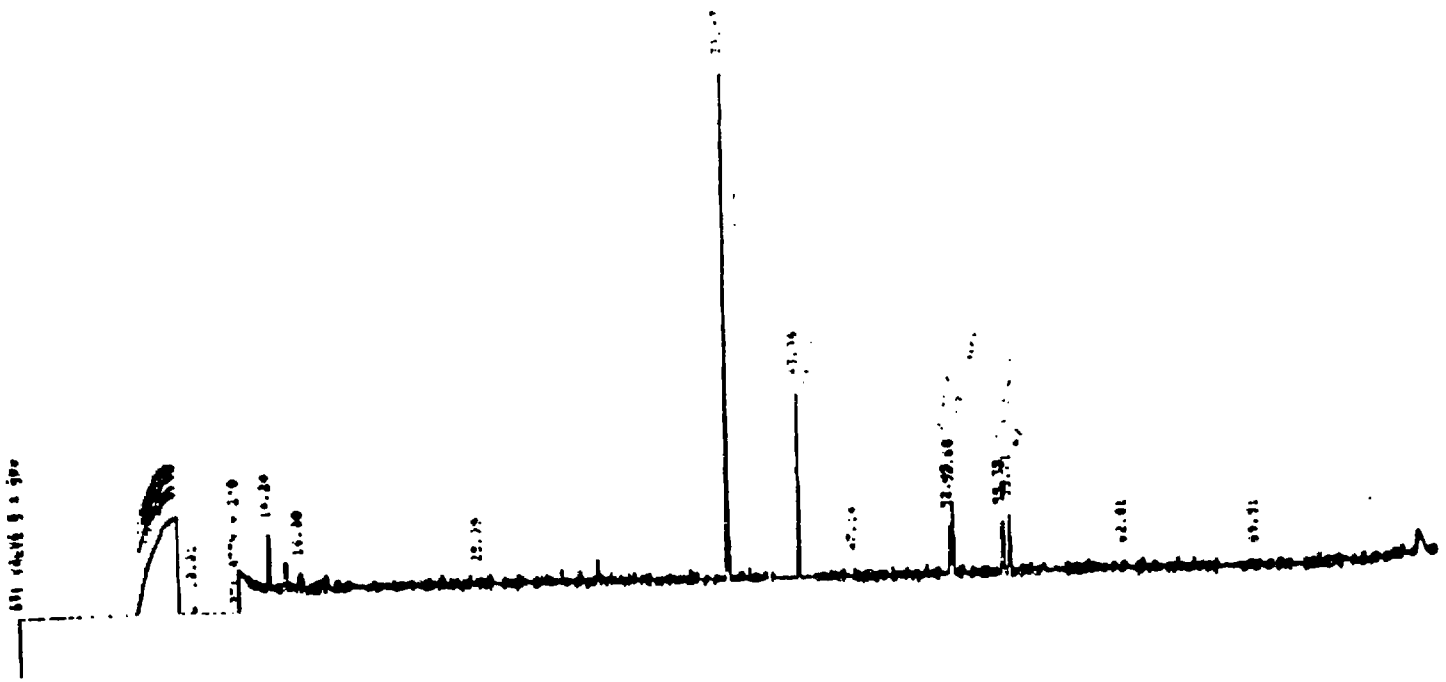

Figure E-9. Gas chromatogram of component(s) extracted from Core 13, position 2 on day 103.

Figure E-10. Gas chromatogram of component(s) extracted from Core 13, position 2 on day 117.

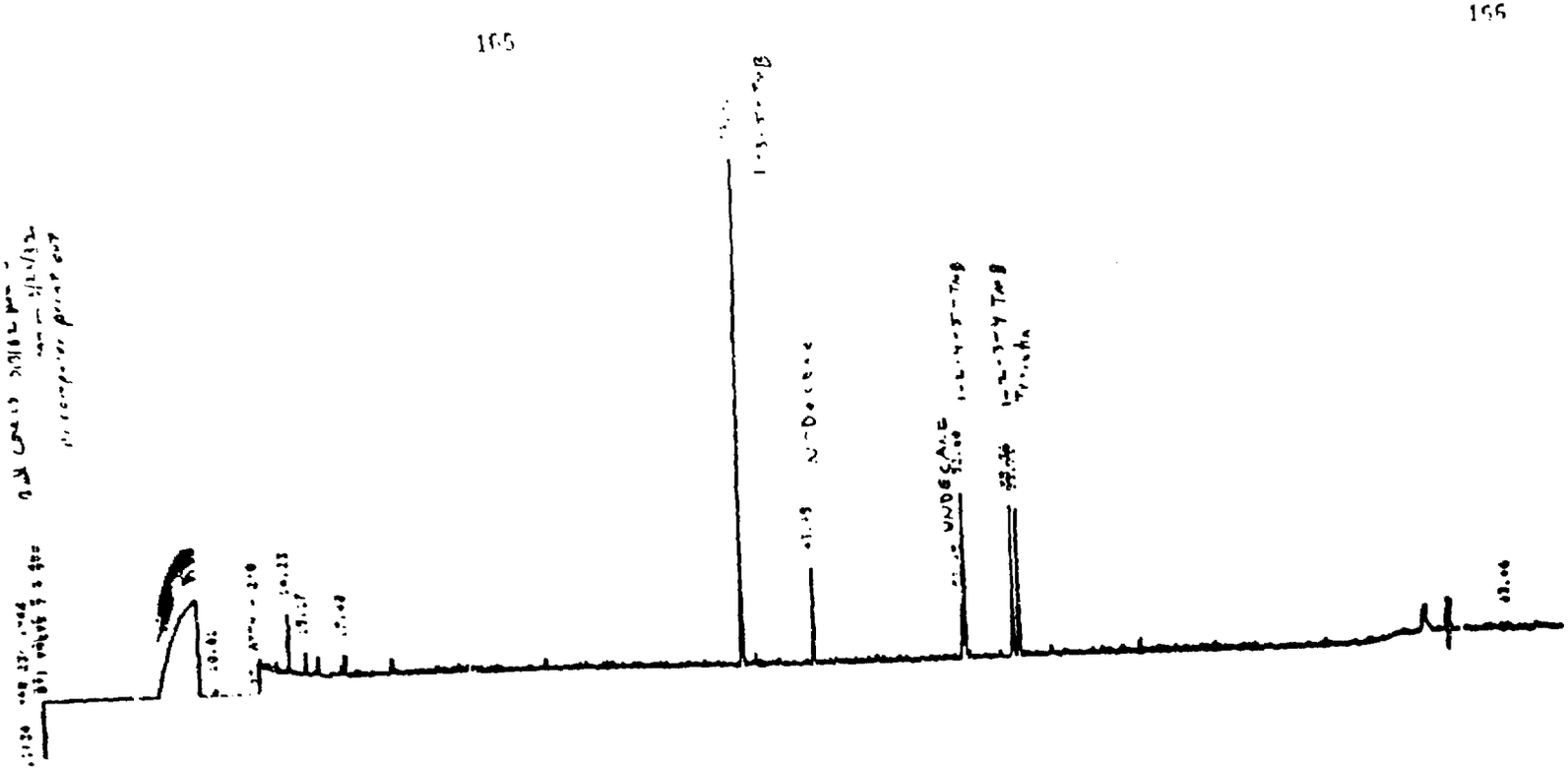

Figure E-11. Gas chromatogram of component(s) extracted from Core 13, position 2 on day 131.

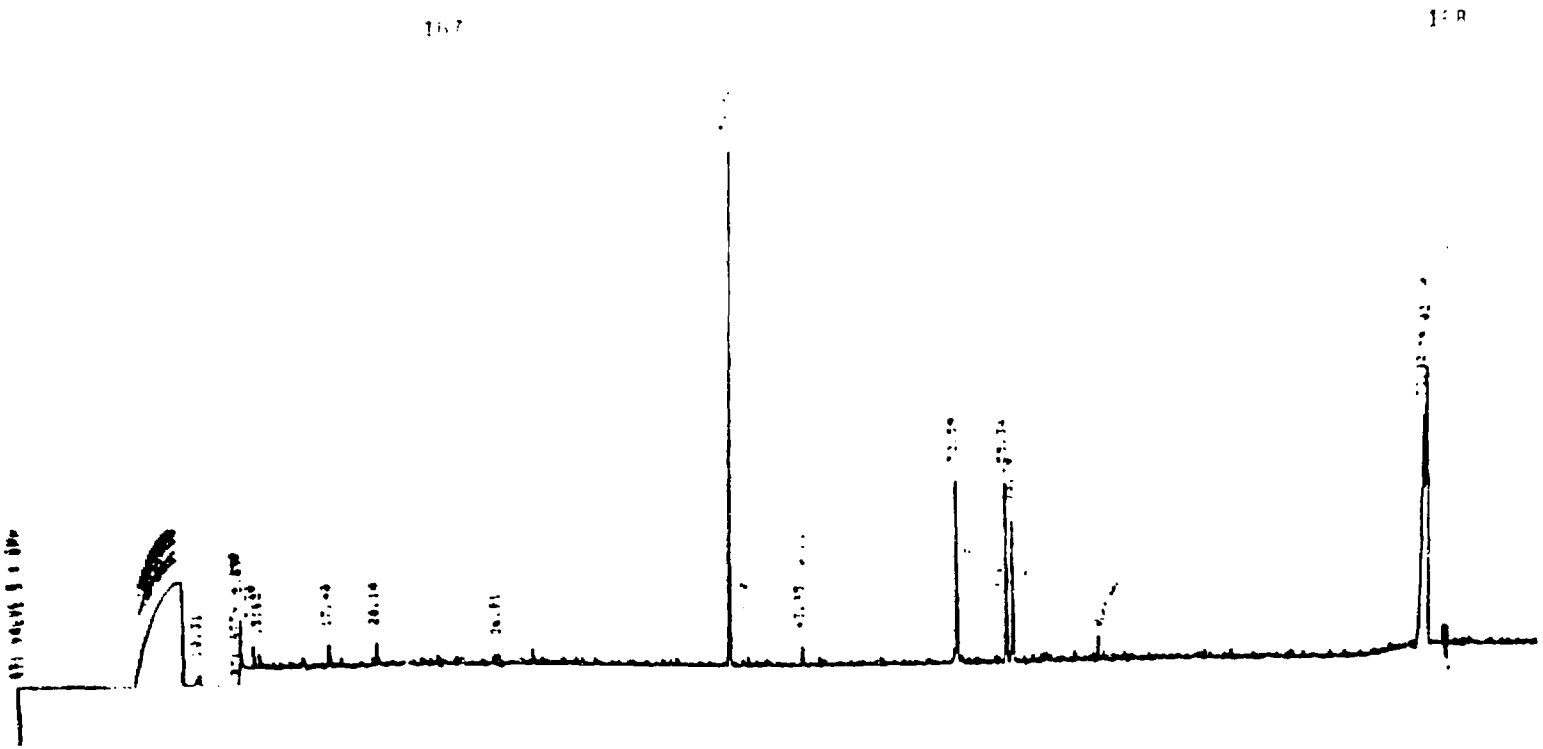

Figure E-12. Gas chromatogram of component(s) extracted from Core 13, position 2 on day 145.

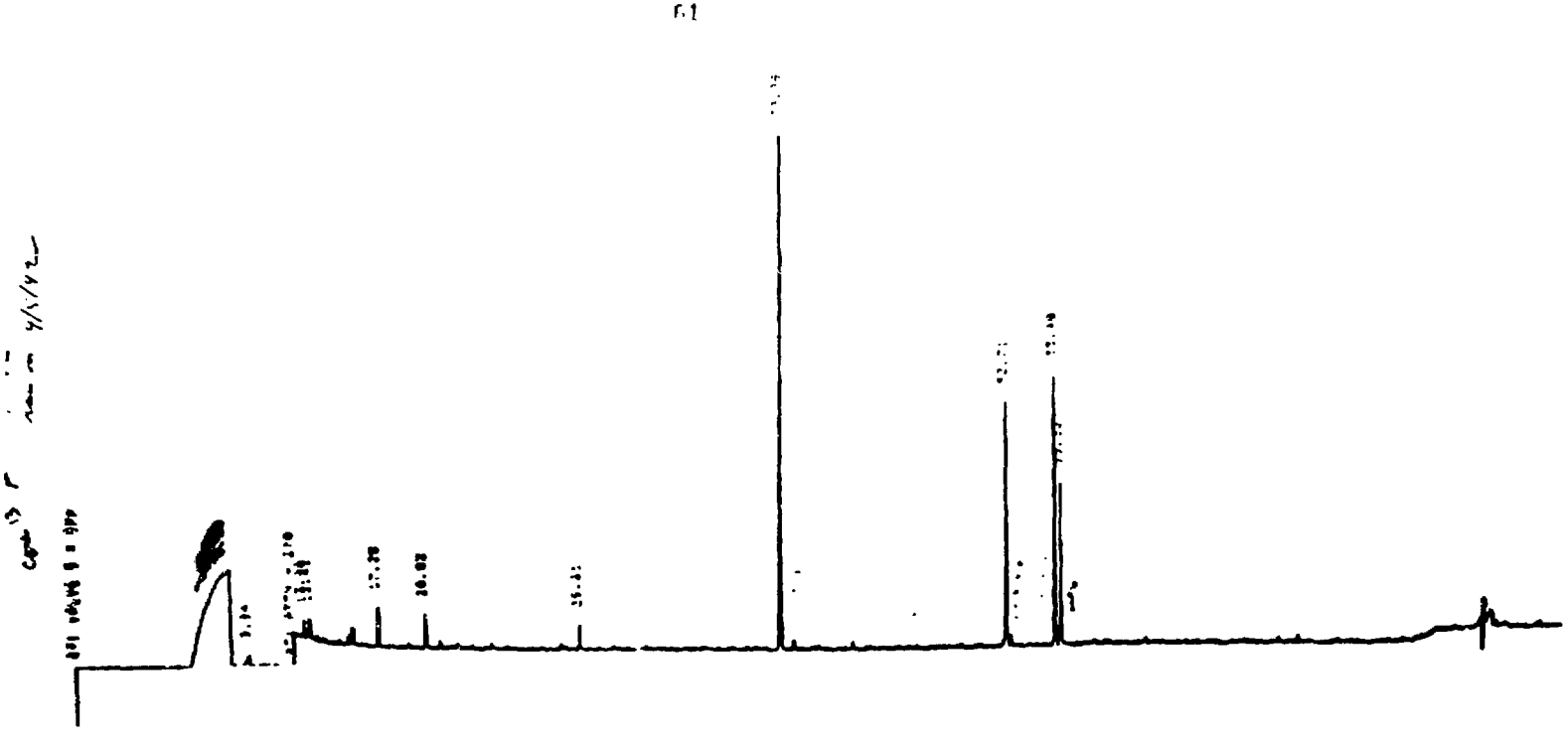

Figure E-13. Gas chromatogram of component(s) extracted from Core 13, position 2 on day 159.

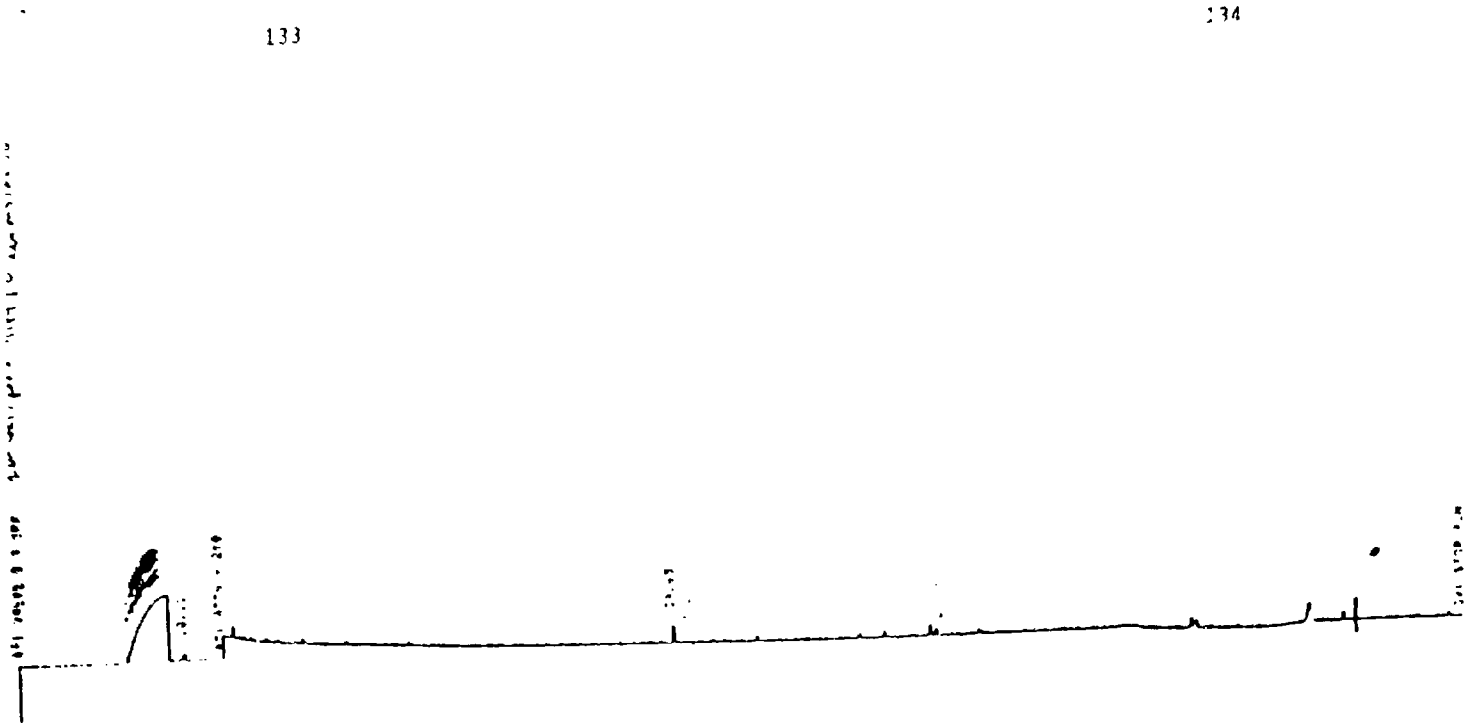

Figure E-14. Gas chromatogram of component(s) extracted from Core 13, position 2 on day 173.

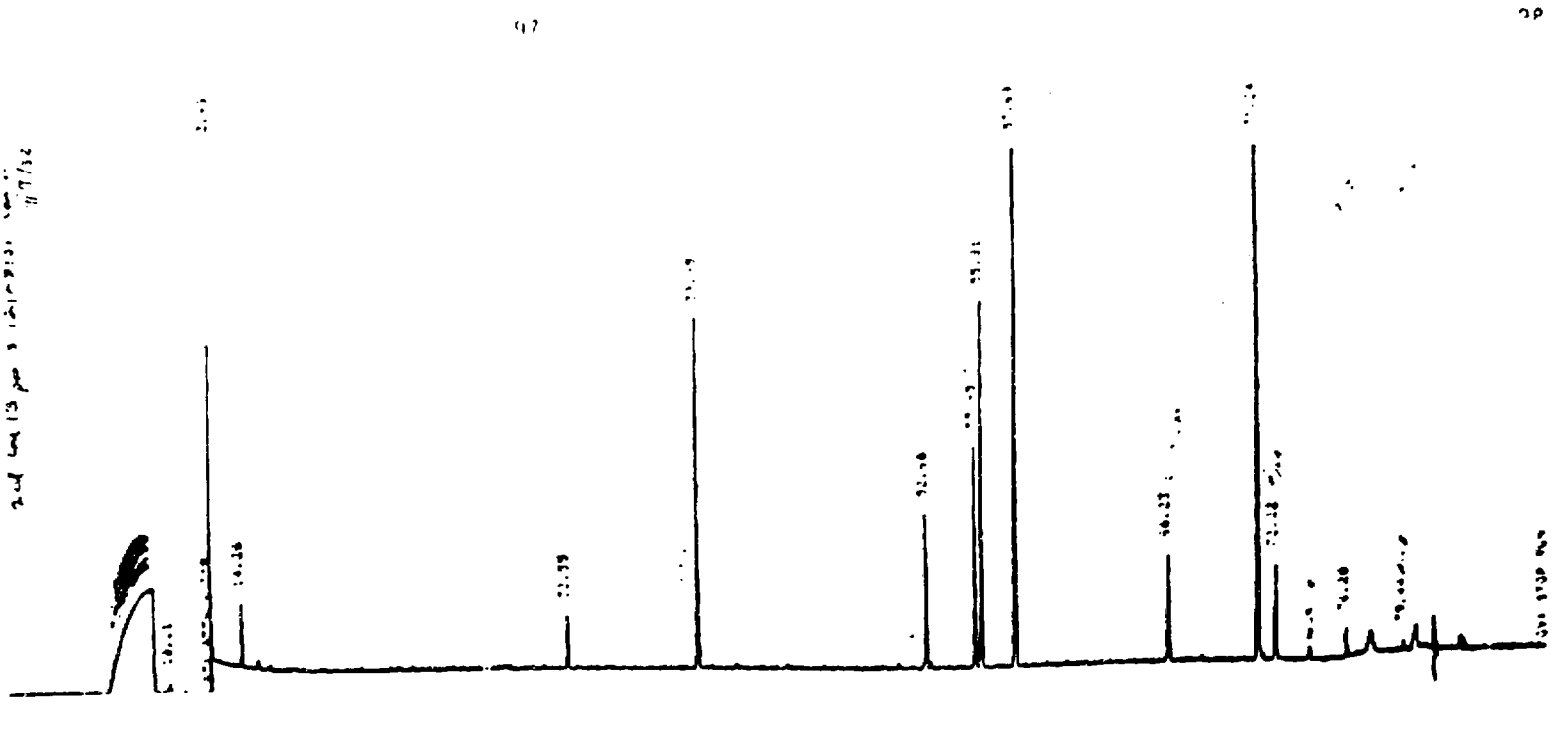

Figure E-15. Gas chromatogram of component(s) extracted from Core 13, position 3 on day 62.

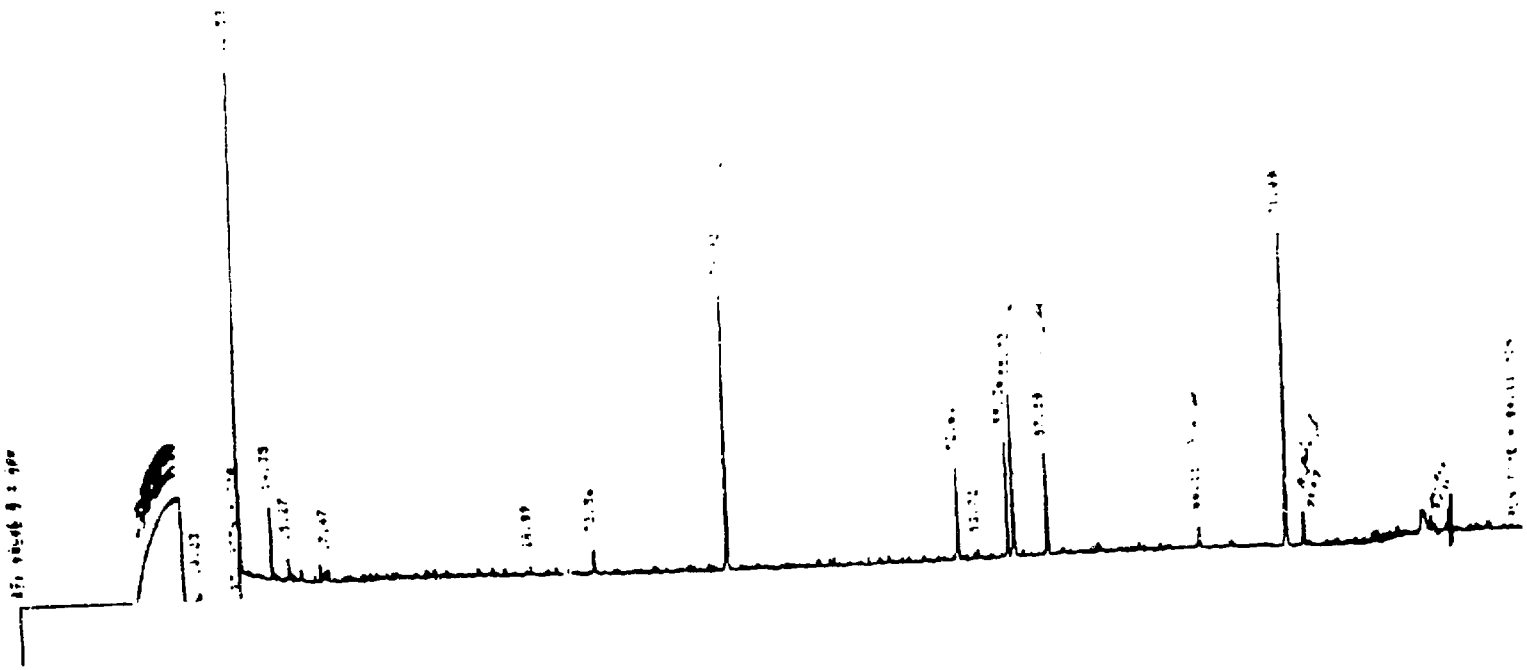

Figure E-16. Gas chromatogram of component(s) extracted from Core 13, position 3 on day 75.

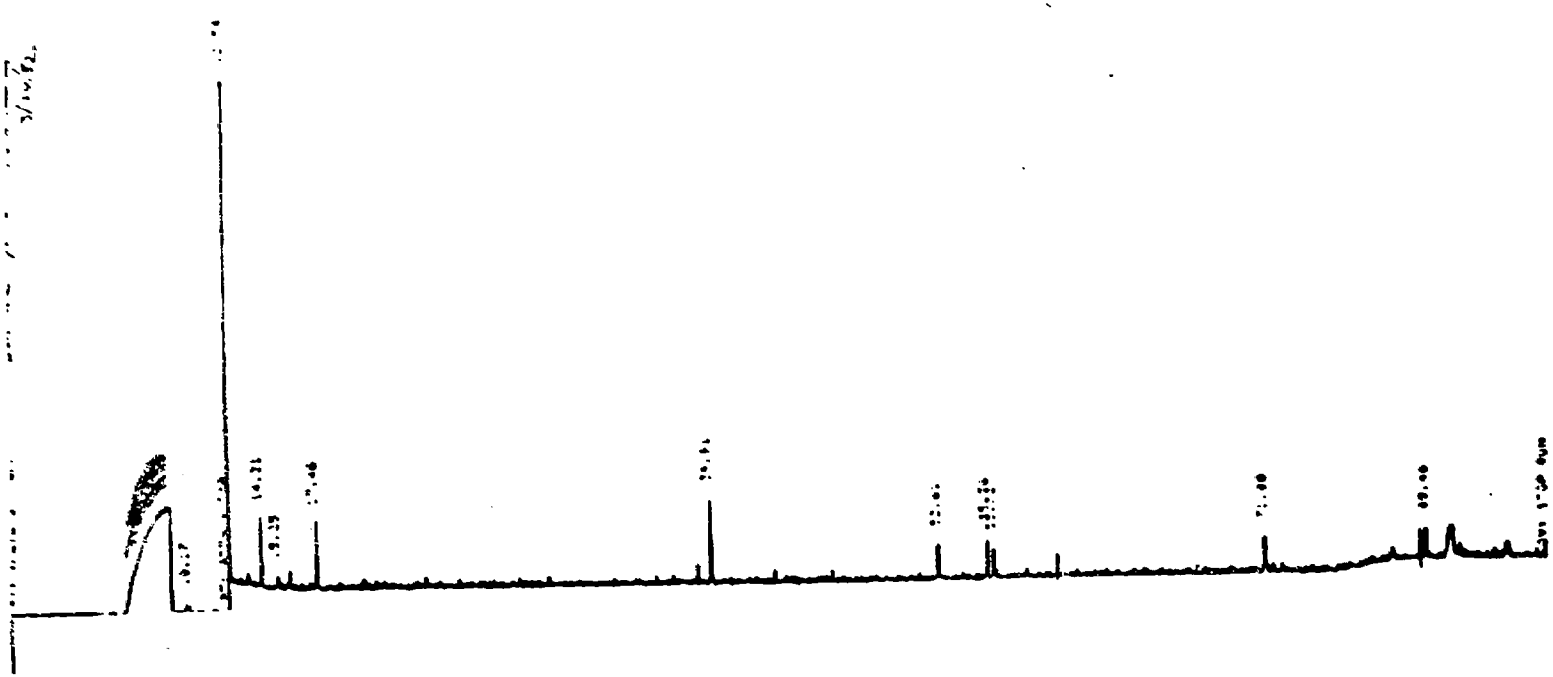

Figure E-17. Gas chromatogram of component(s) extracted from Core 13, position 3 on day 103.

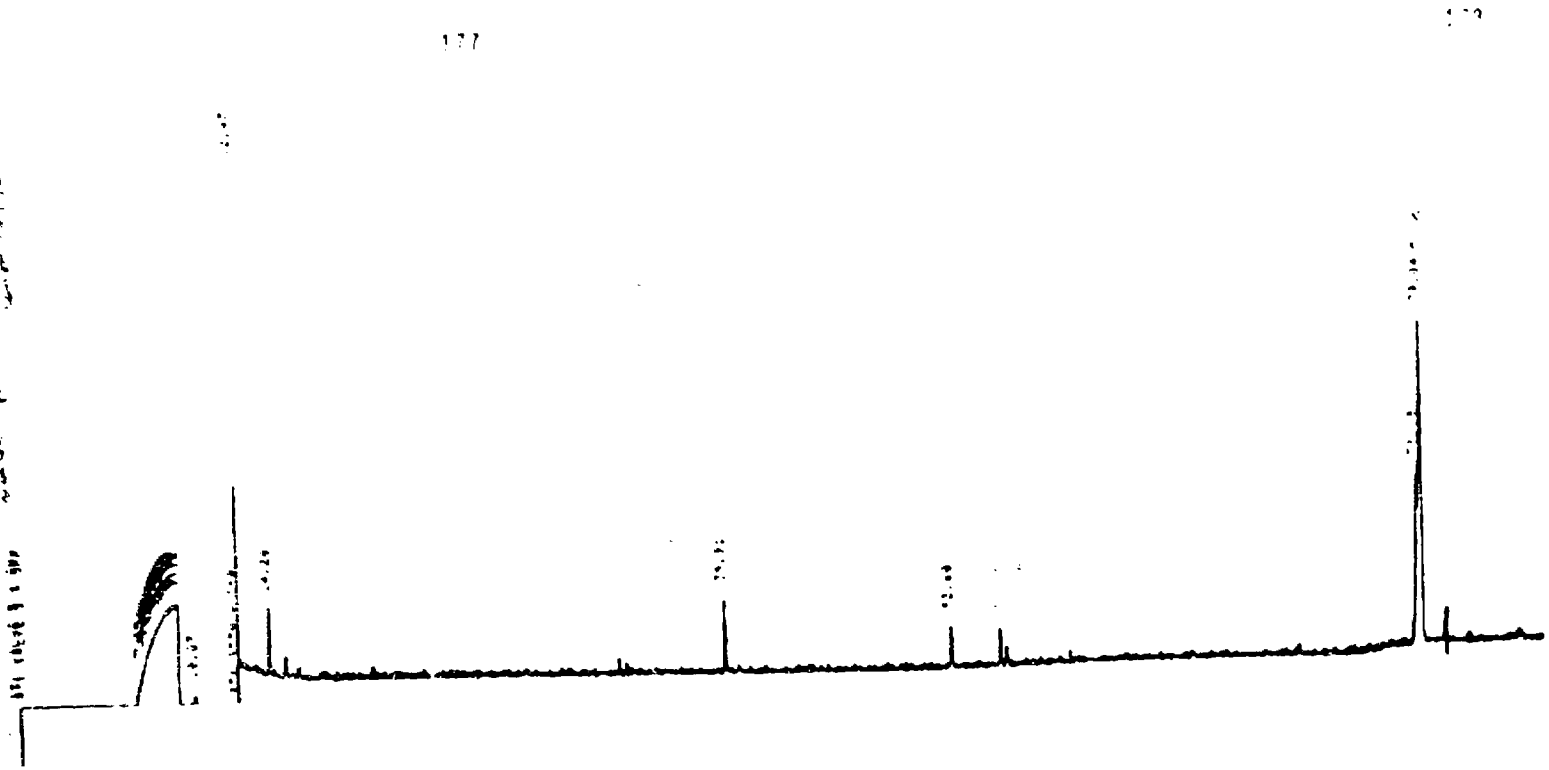

Figure E-18. Gas chromatogram of component(s) extracted from Core 13, position 3 on day 131.

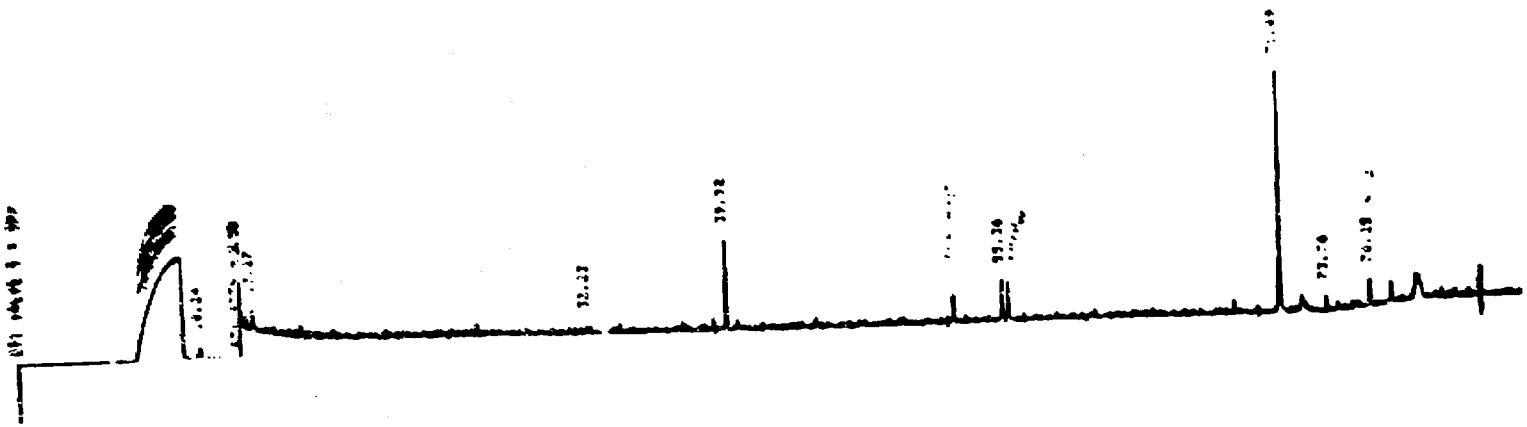

Figure E-19. Gas chromatogram of component(s) extracted from Core 13, position 3 on day 145.

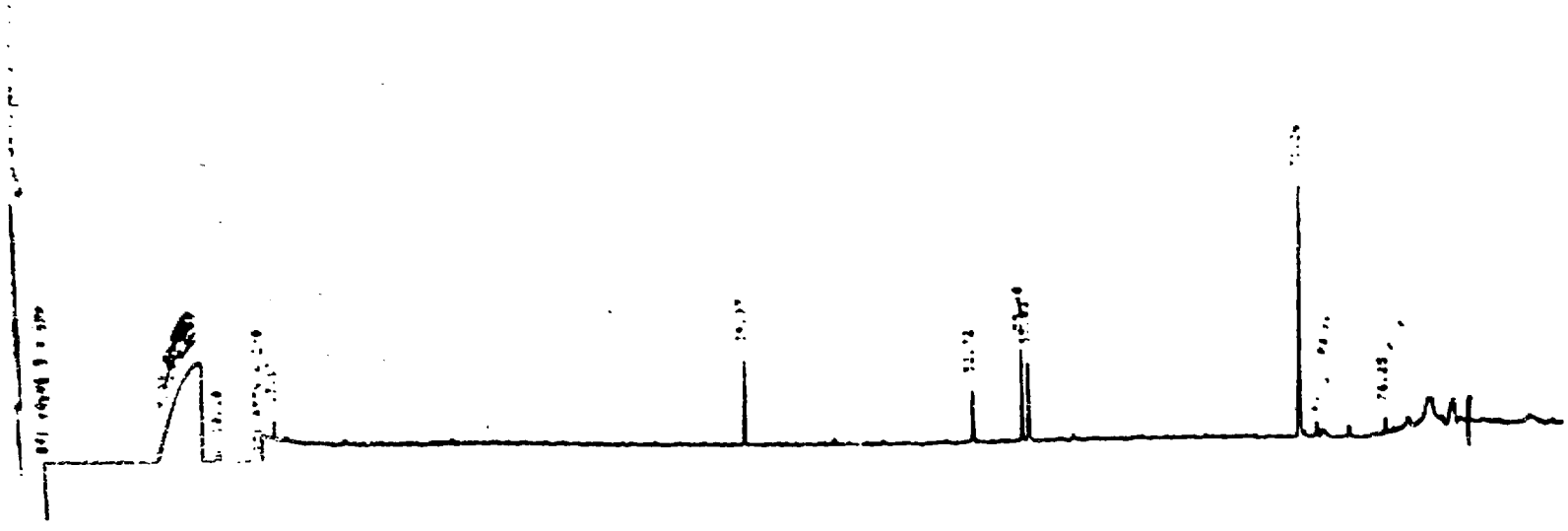

Figure E-20. Gas chromatogram of component(s) extracted from Core 13, position 3 on day 159.

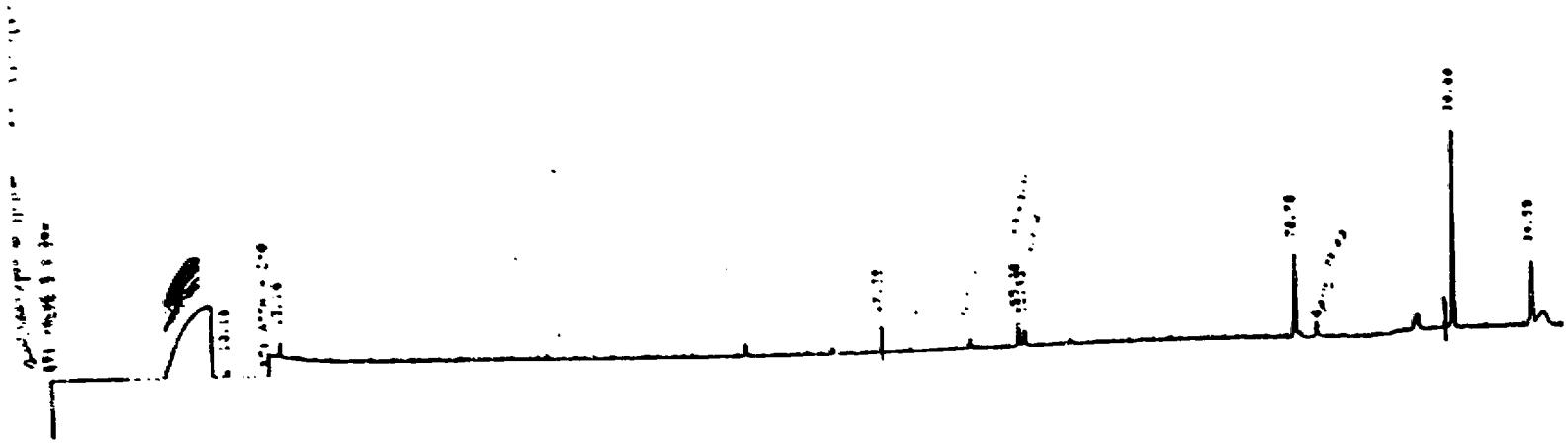

Figure E-21. Gas chromatogram of component(s) extracted from Core 13, position 3 on day 173.

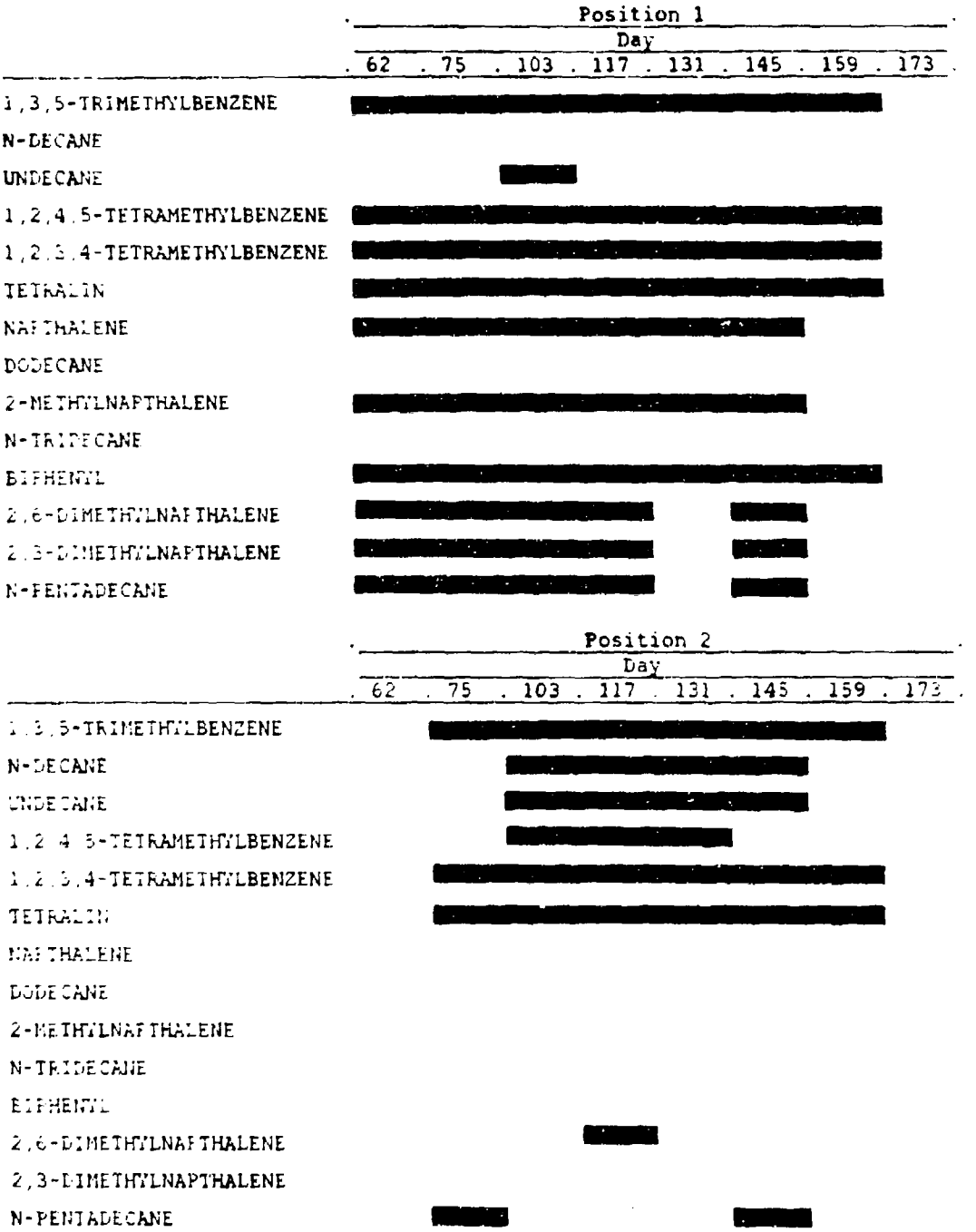

Figure E-22. Transport of model JP-5 components in Core 13* over time.

*Core 13 was studied in outdoor laboratory.

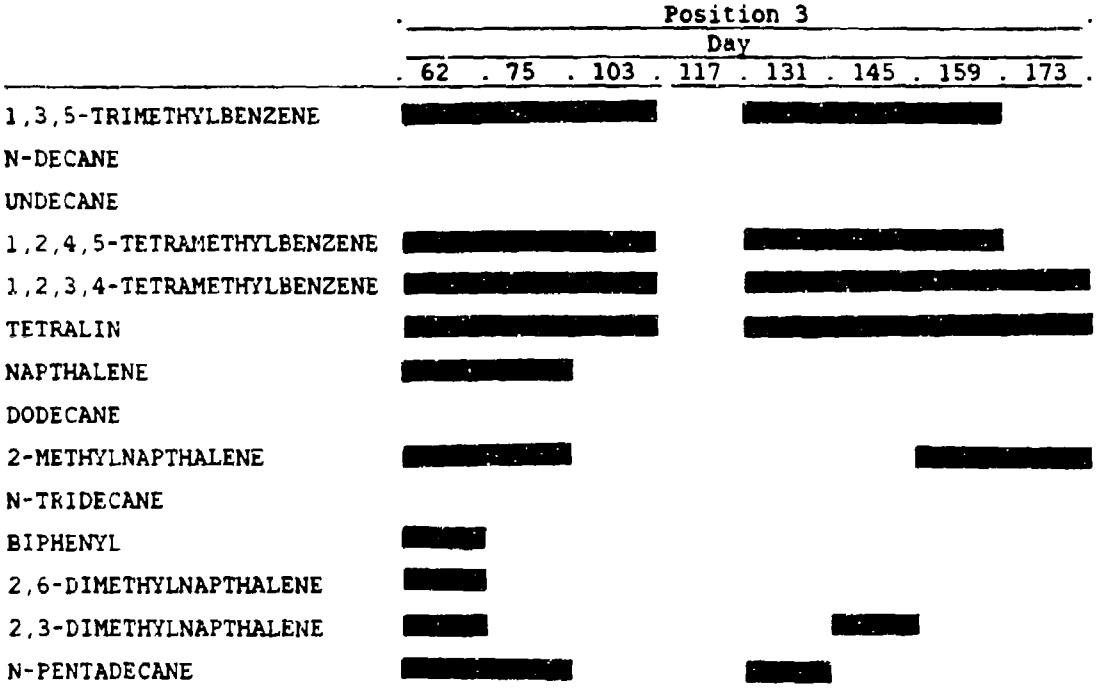

Figure E-22 (continued)

TABLE E-1. TRANSPORT SUMMARY OF MODEL JP-5 THROUGH OUTDOOR CORE 13[a]

Probe position	Day										
	62	75	103	117	131	145	159	173	187	201	214
1	10	10	13	10	7	10	5	0	-	-	-
2	-	2	6	7	6	6	4	0	-	-	-
3	10	8	6	0	5	5	5	3	-	-	-
4	-	0	0	0	0	0	0	0	-	-	-
5	-	0	0	0	0	0	0	0	0	-	-
Bottom	0	0	0	0	0	0	0	0	0	0	0

[a]Values reported represent the number of peaks identified by GC analysis. Dashes indicate that no GC analysis was run on that day.

APPENDIX F

TRANSPORT OF MODEL JP-5 IN SMALL CORES

Figure F-1. Gas chromatogram of component(s) extracted from Core 21 on day 14.

Figure F-2. Gas chromatogram of component(s) extracted from Core 21 on day 28.

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Figure F-3. Gas chromatogram of component(s) extracted from Core 22 on day 28.

APPENDIX G

HEADSPACE GASES

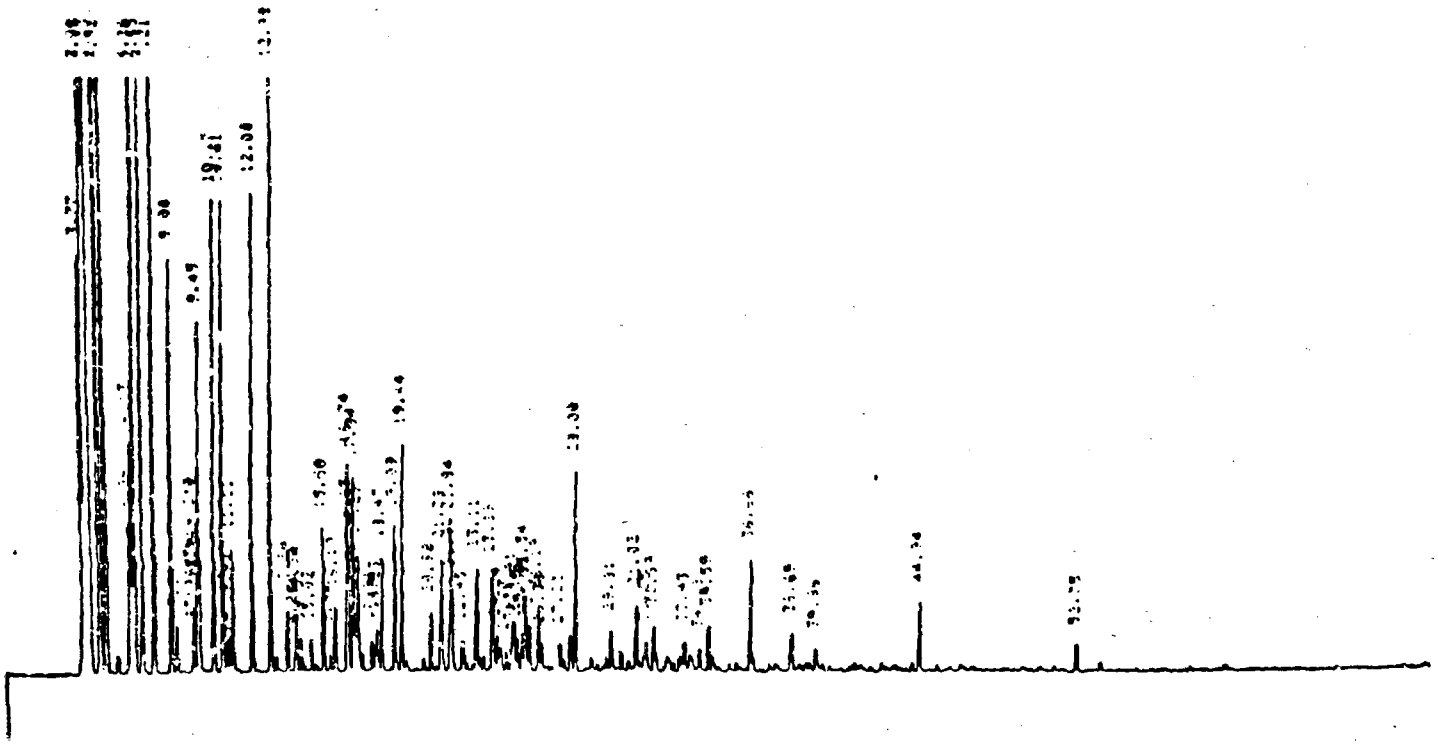

Figure G-1. Gas chromatogram of vapors from above shale-derived JP-4 standard.

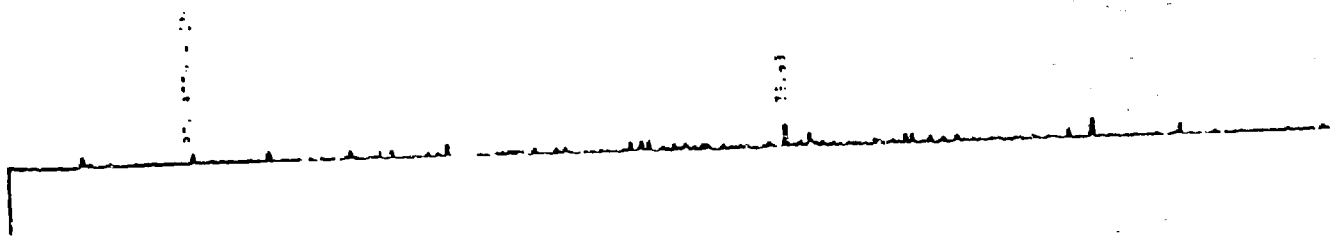

Figure G-2. Gas chromatogram of headspace gases from a JP-4 treated core using a gas syringe.

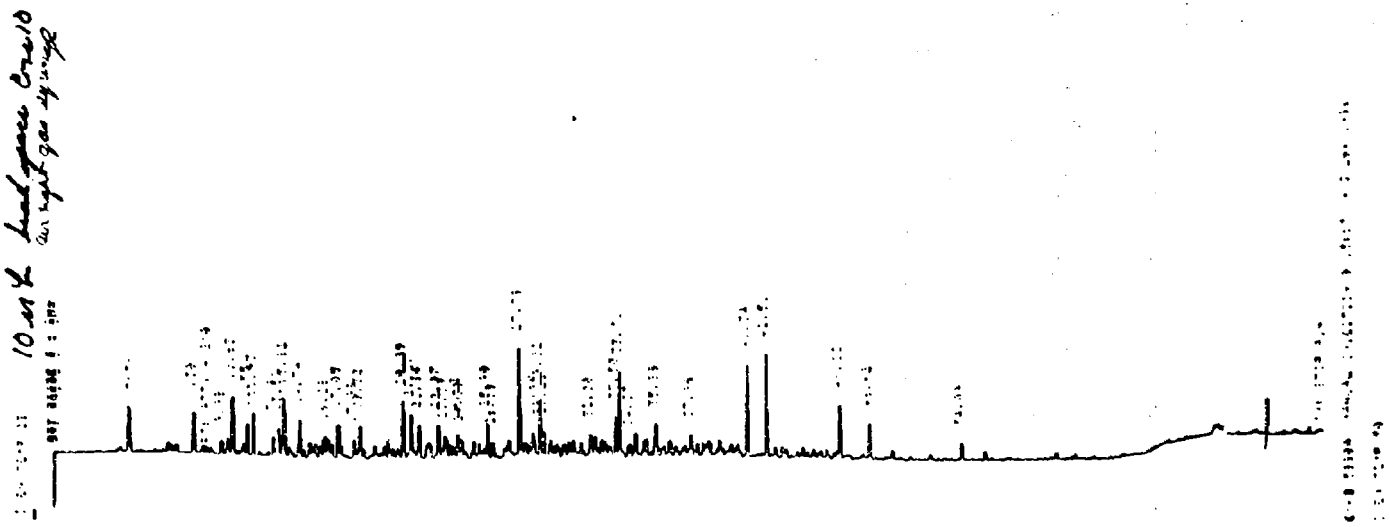

Figure G-3. Gas chromatogram of headspace gases from a JP-4 treated core using a leak-tight syringe.

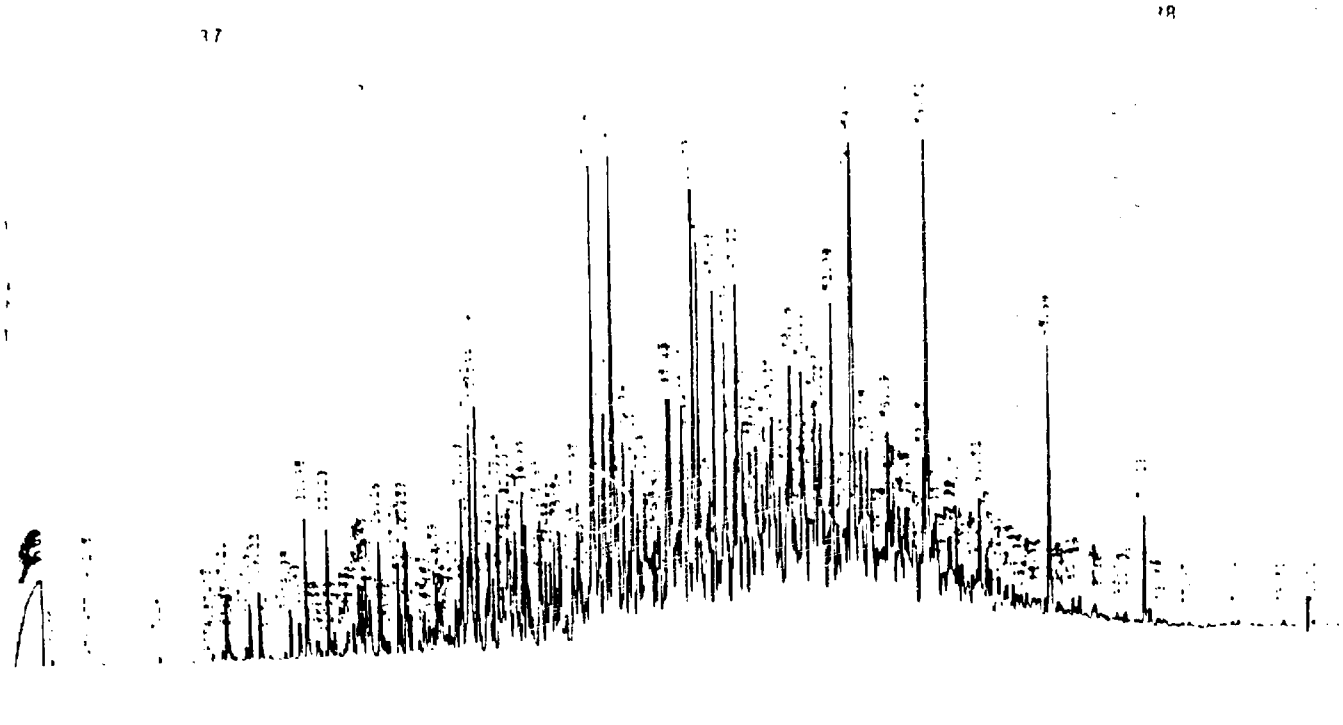

Figure G-4. Gas chromatogram of headspace gases from a JP-5 treated core using a passive dosimeter.

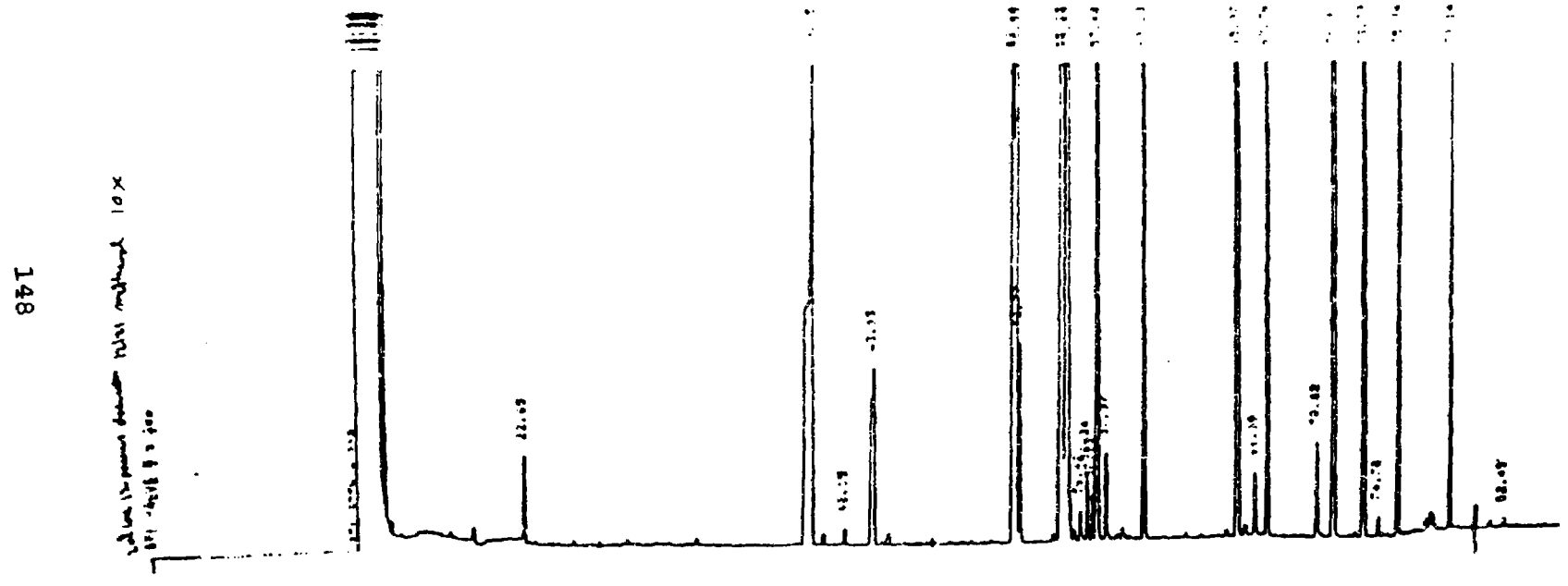

Figure G-5. Gas chromatogram of component(s) extracted with methanol from Core 12's passive dosimeter on day 59.

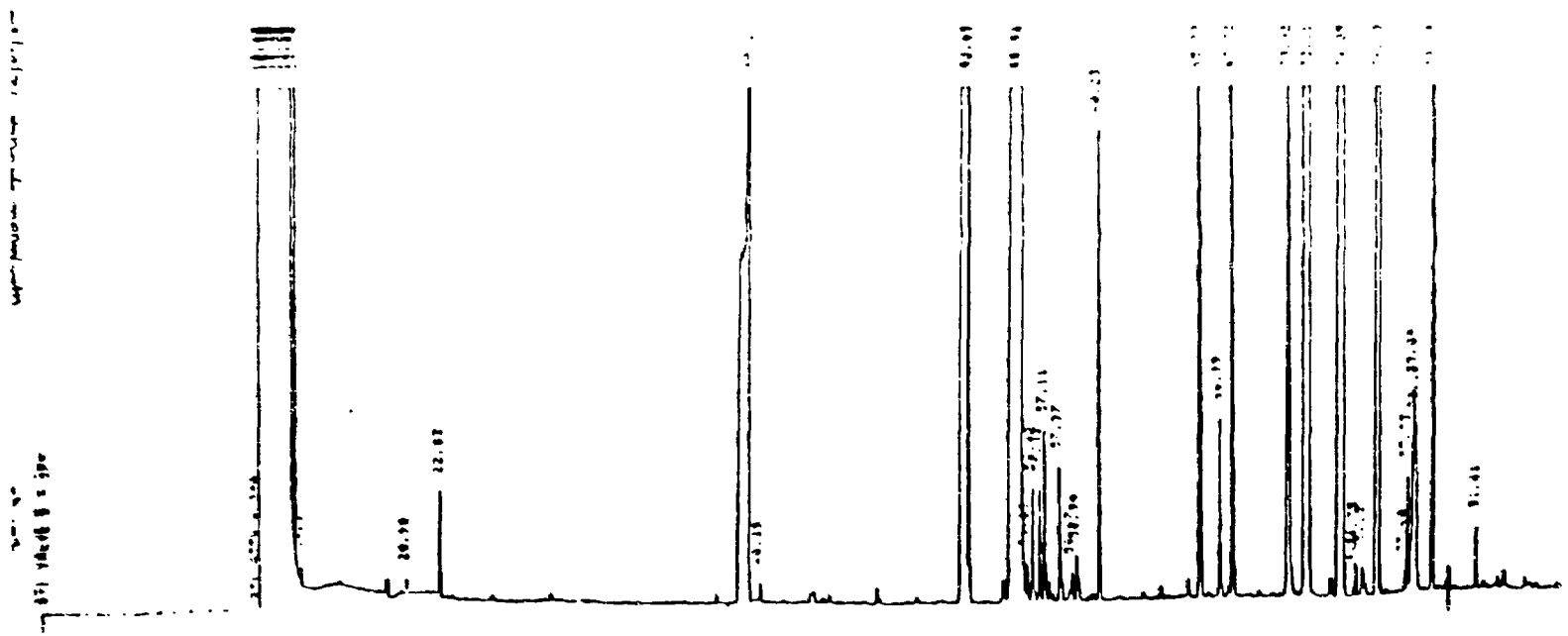

Figure G-6. Gas chromatogram of component(s) extracted with methanol from Core 12's passive dosimeter on day 60.

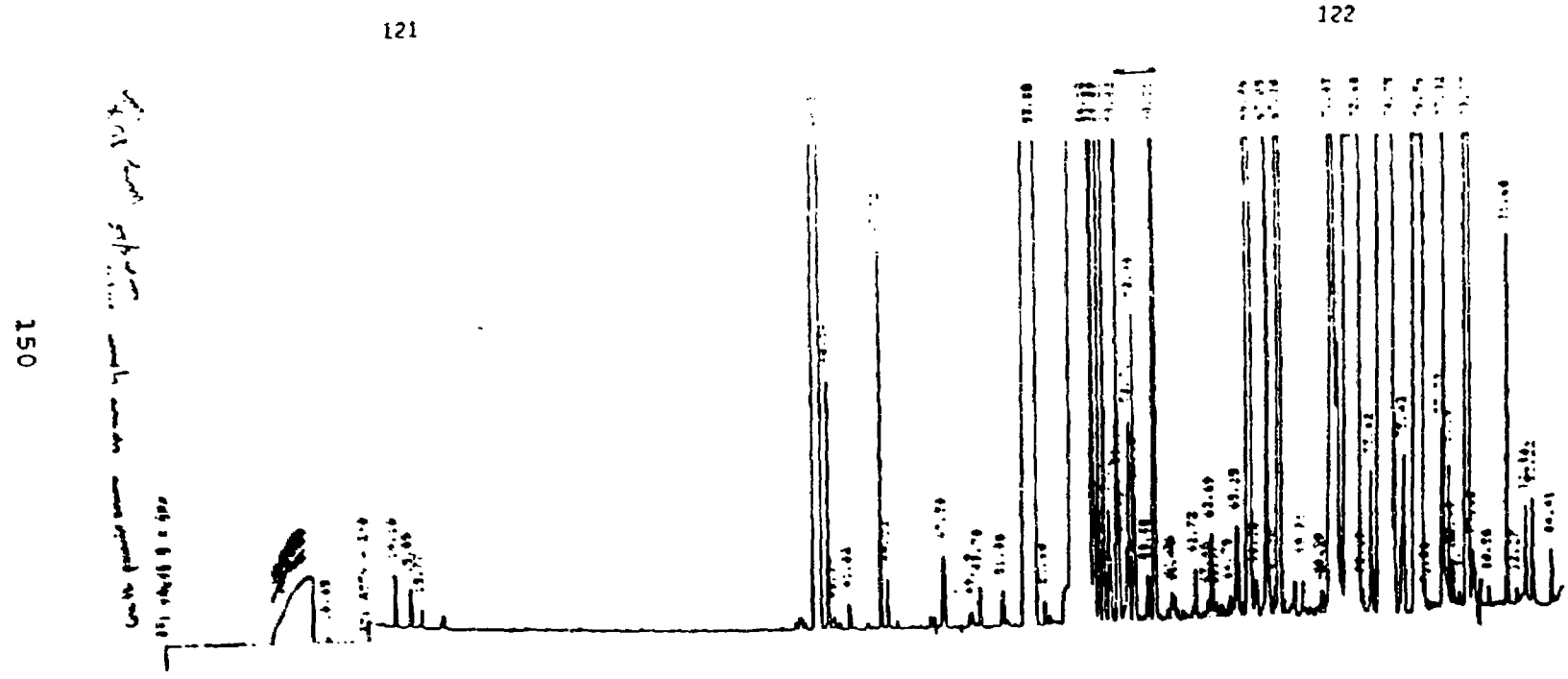

Figure G-7. Gas chromatogram of component(s) extracted with pentane from Core 12's passive dosimeter on day 60.

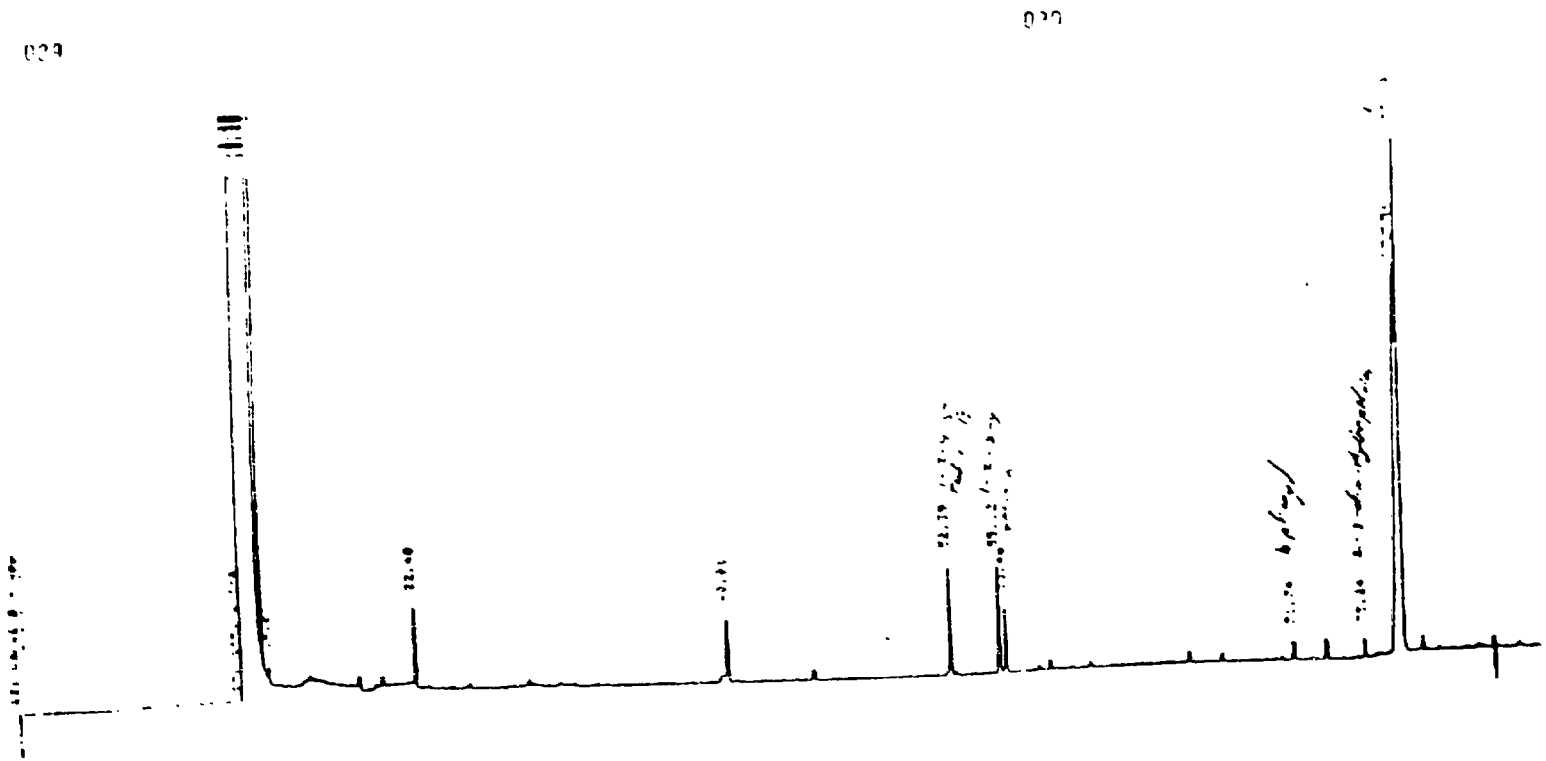

Figure G-8. Gas chromatogram of component(s) extracted with methanol from Core 11's passive dosimeter on day 60.

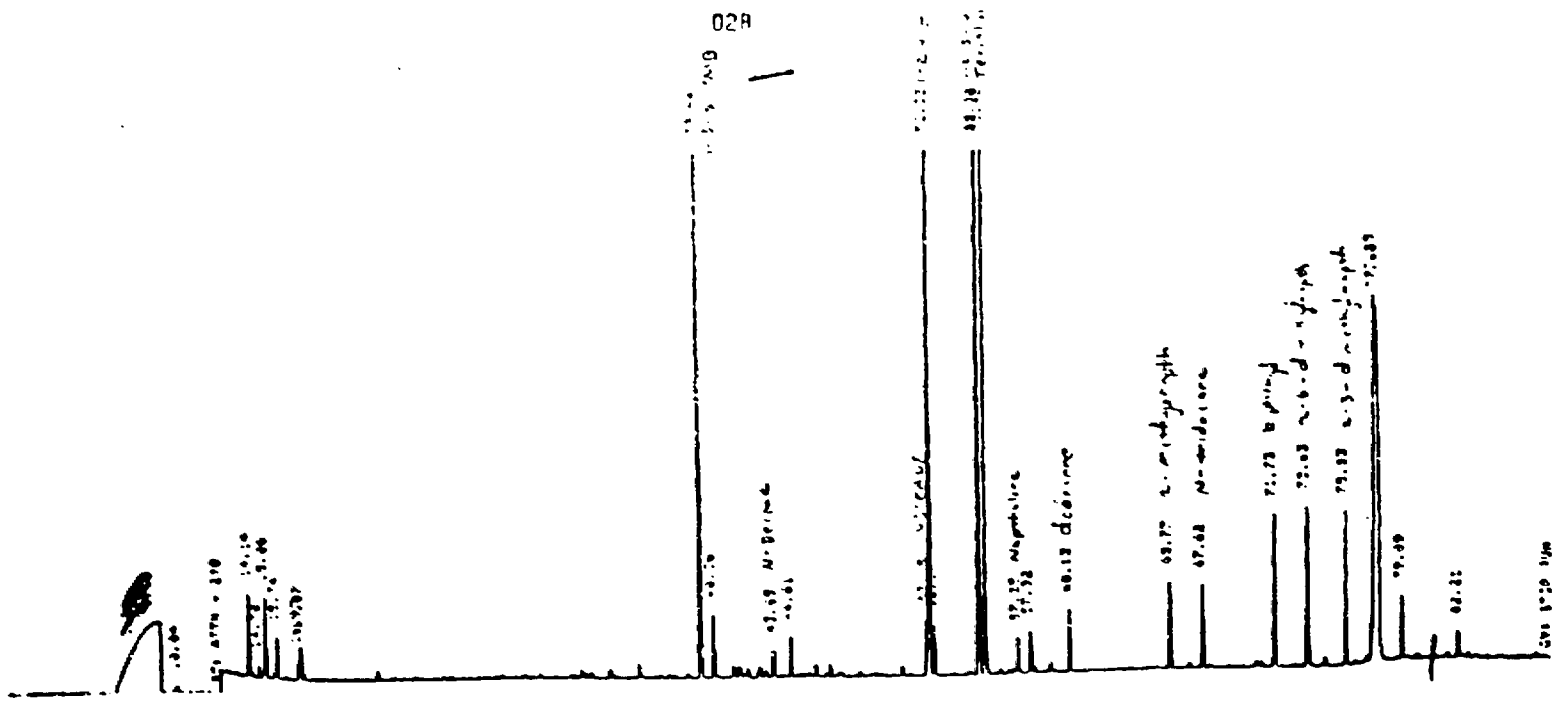

Figure G-9. Gas chromatogram of component(s) extracted with pentane from Core 11's passive dosimeter on day 60.

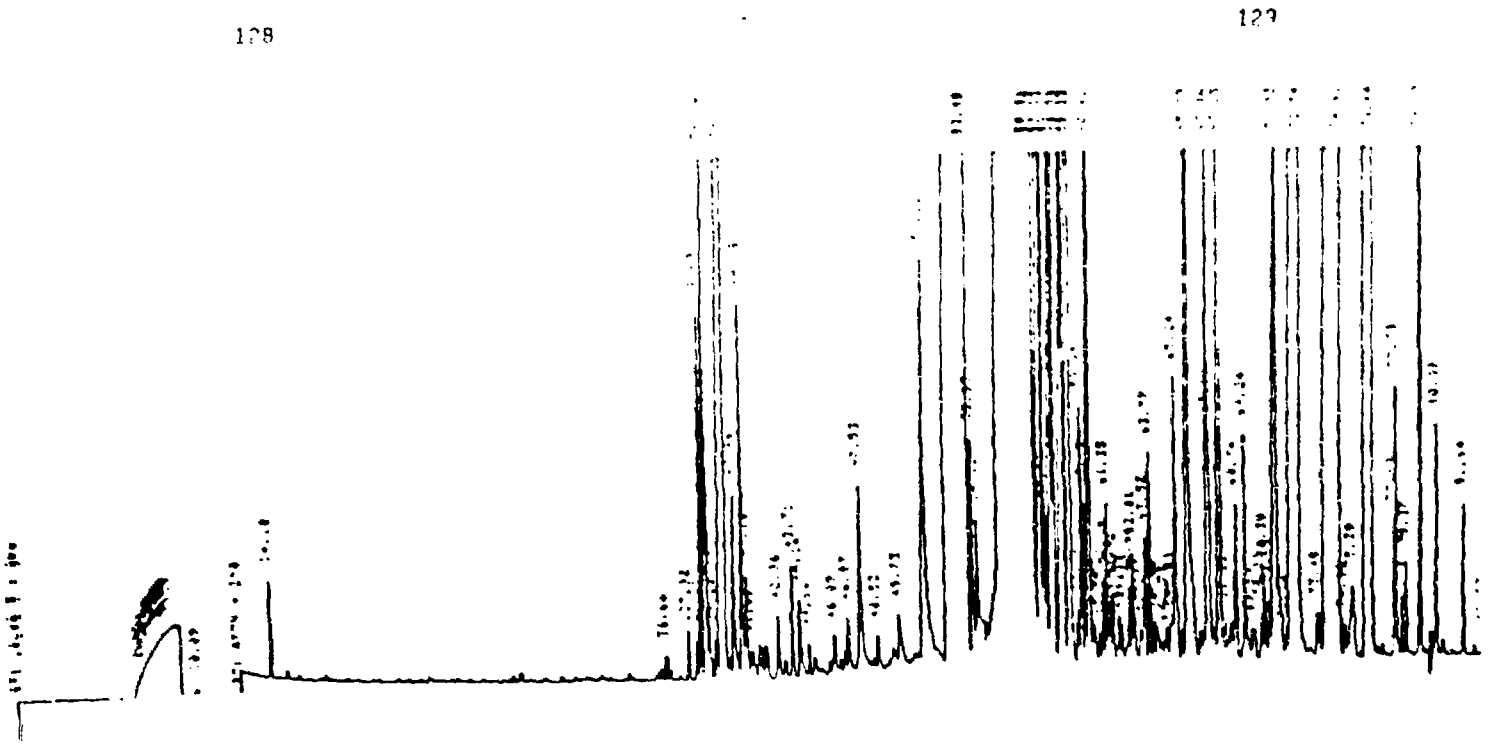

Figure G-10. Gas chromatogram of component(s) extracted with methanol from Core 12's passive dosimeter on day 75.

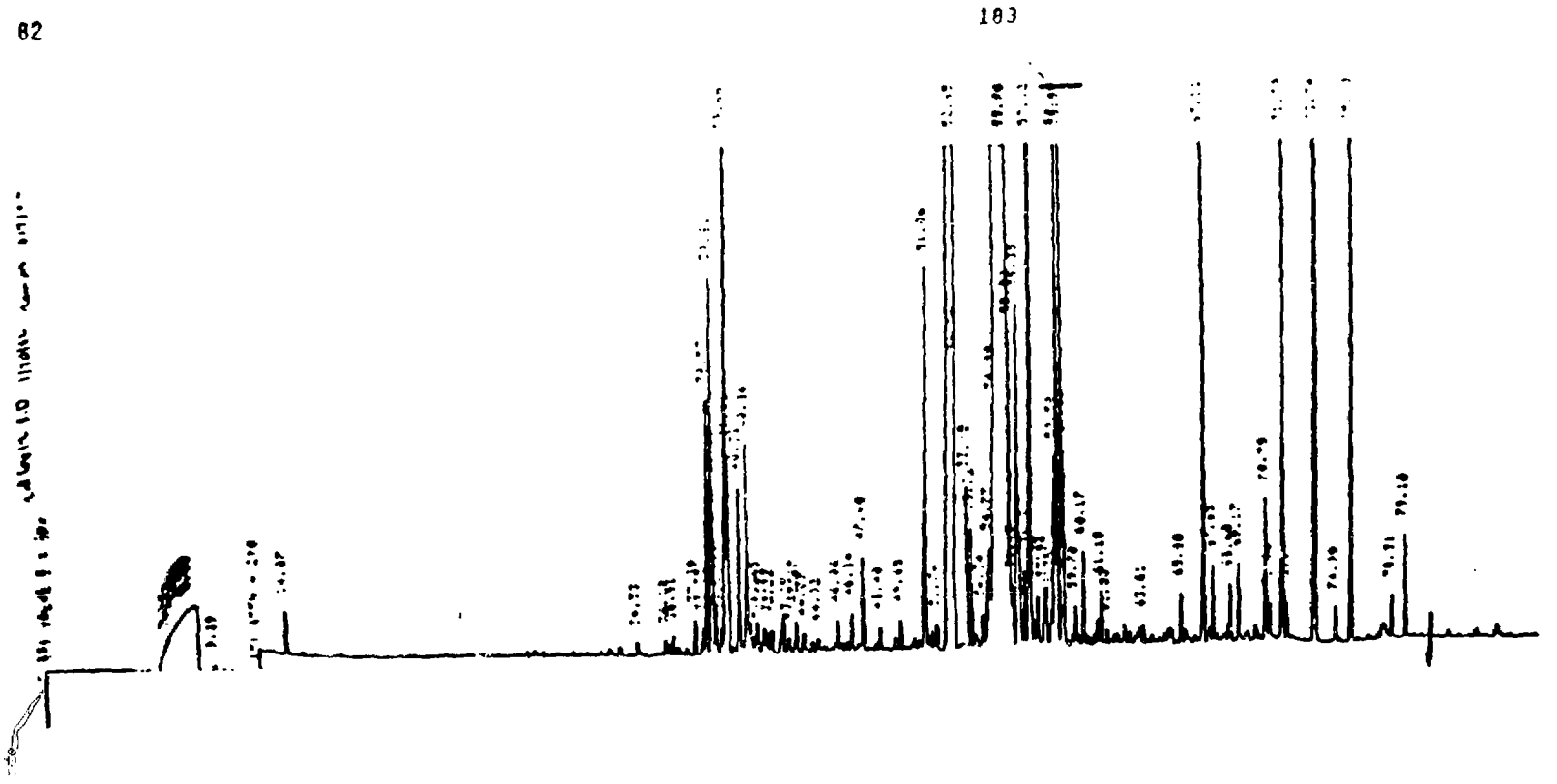

Figure G-11. Gas chromatogram of component(s) extracted with pentane from Core 12's passive dosimeter on day 89.

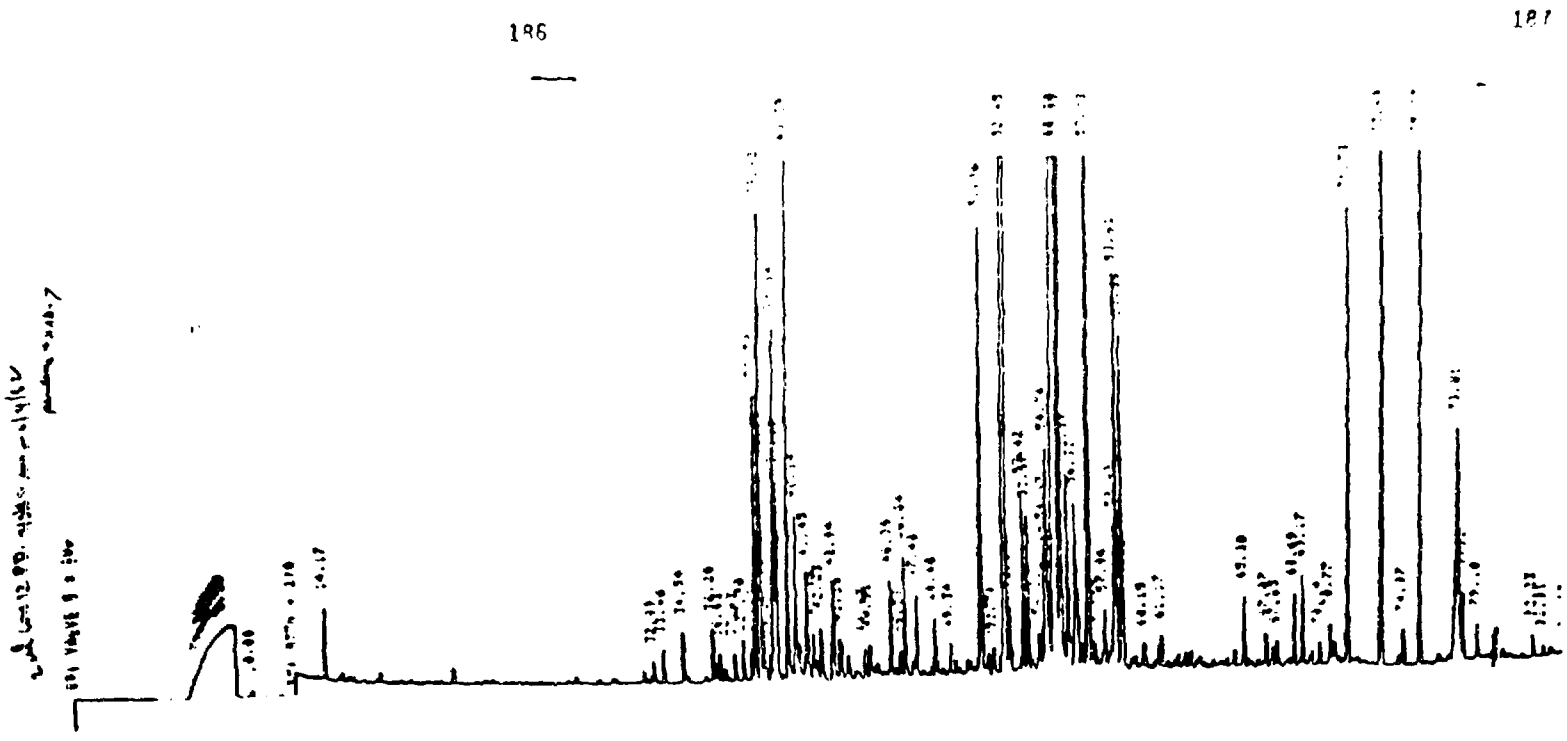

Figure G-12. Gas chromatogram of component(s) extracted with pentane from Core 12's passive dosimeter on day 103.

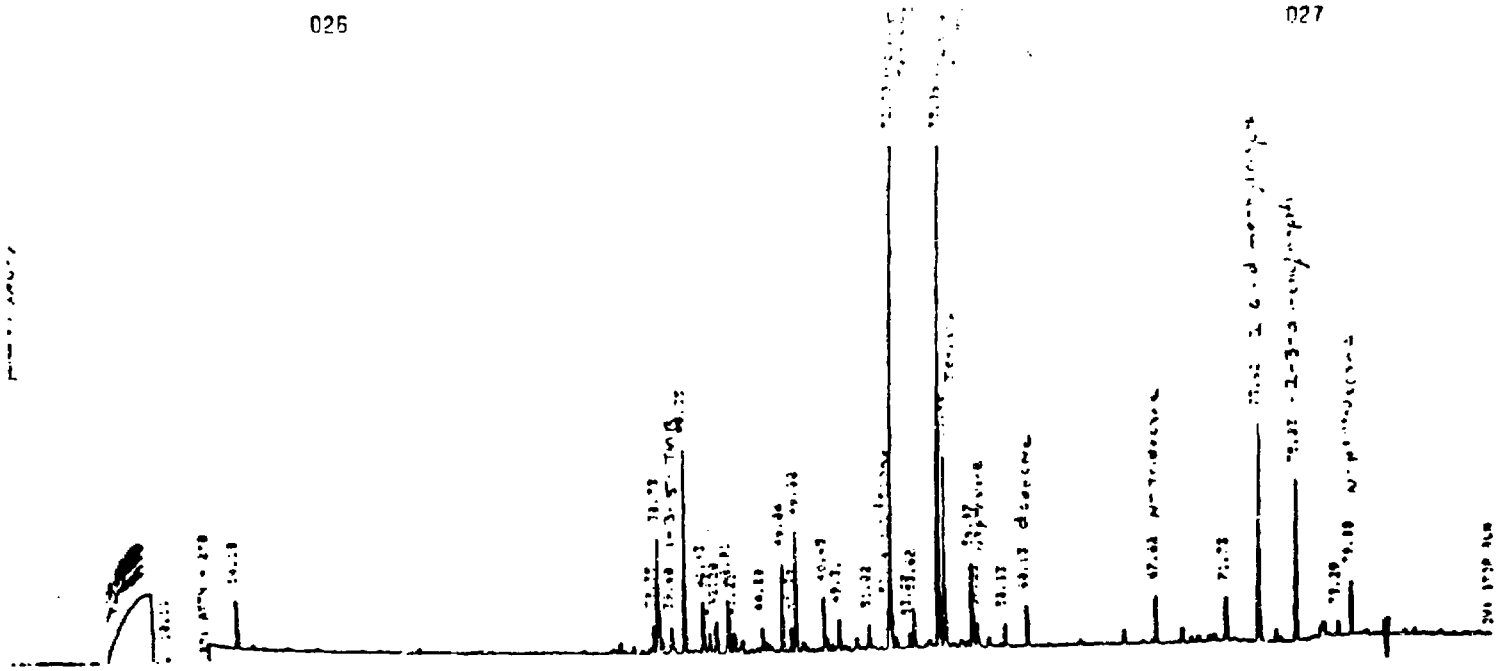

Figure G-13. Gas chromatogram of component(s) extracted with pentane from Core 12's passive dosimeter on day 117.

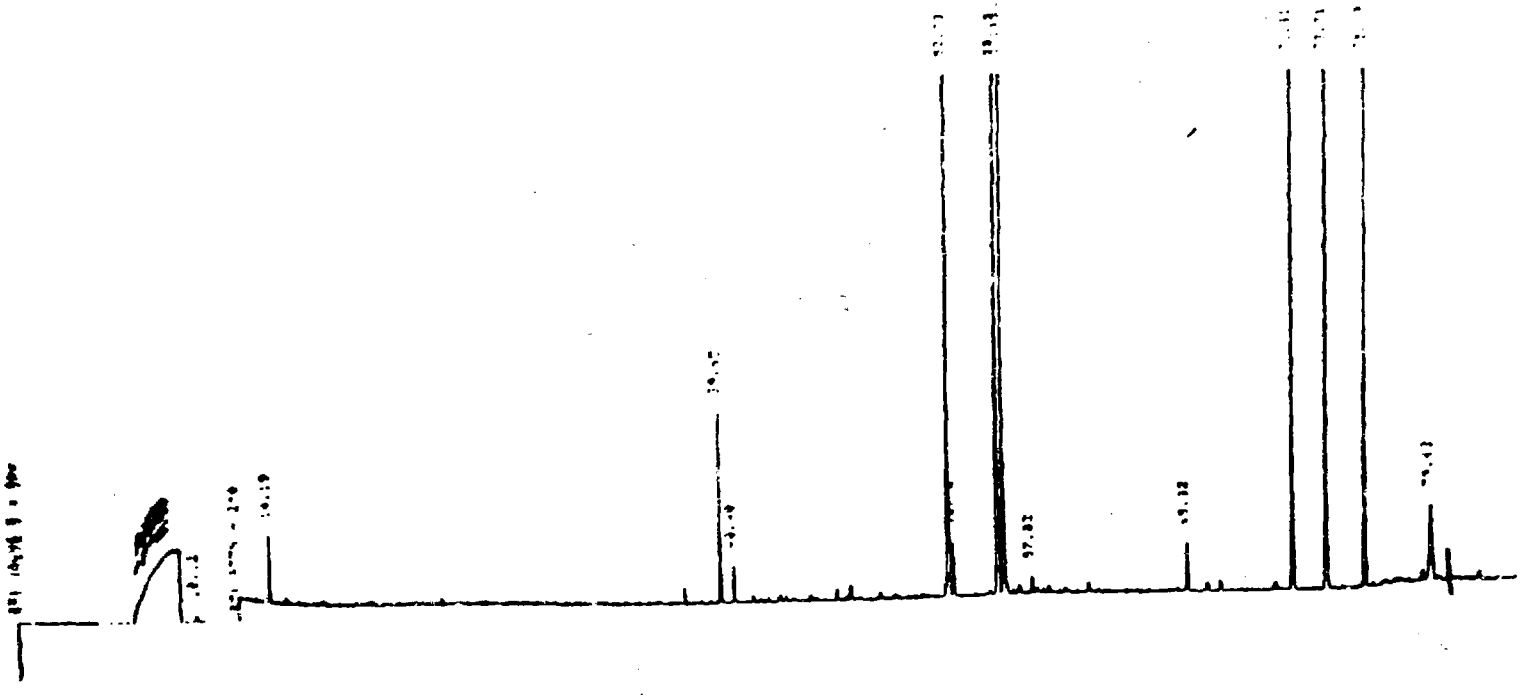

Figure G-14. Gas chromatogram of component(s) extracted with pentane from Core 11's passive dosimeter on day 75.

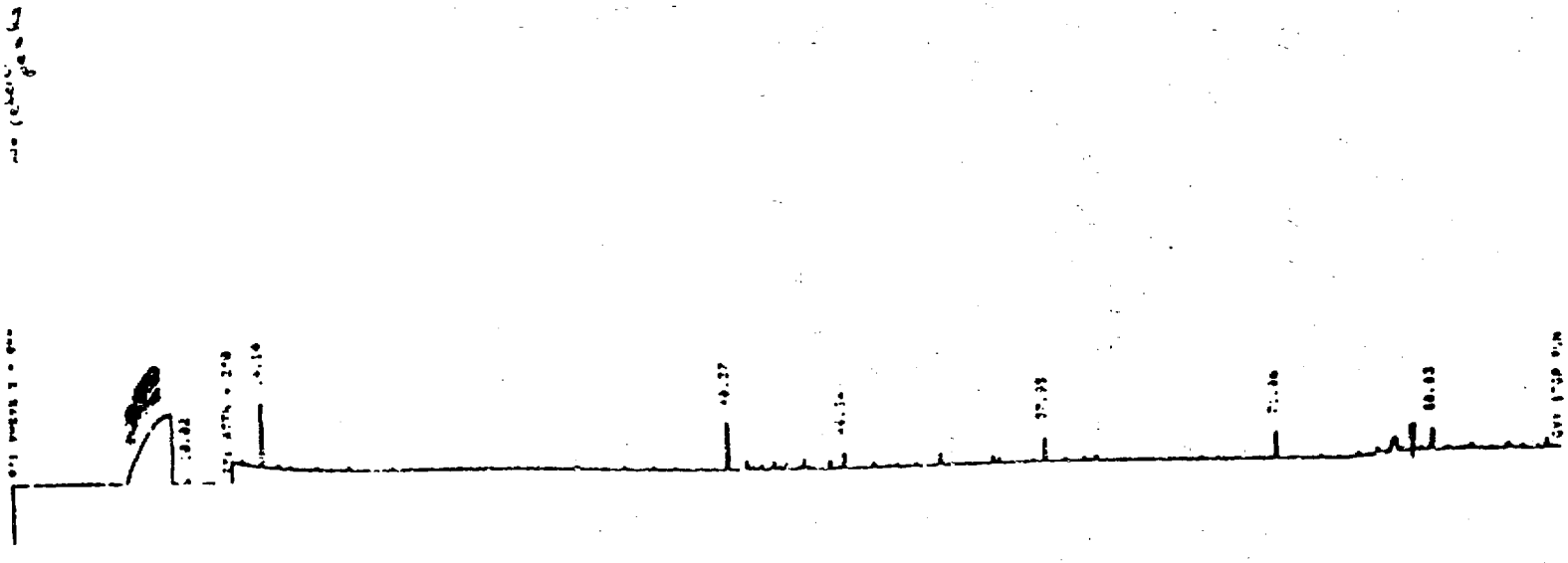

Figure G-15. Gas chromatogram of component(s) extracted with pentane from Core 11's passive dosimeter on day 89.

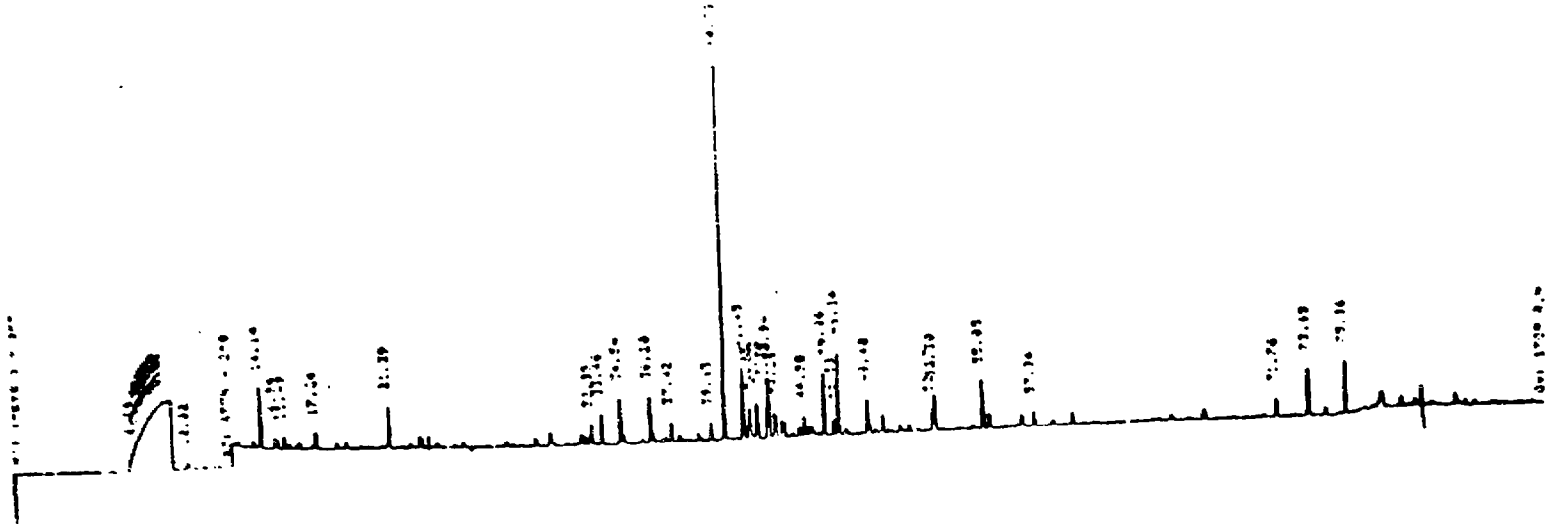

Figure G-16. Gas chromatogram of component(s) extracted with pentane from Core 11's passive dosimeter on day 103.

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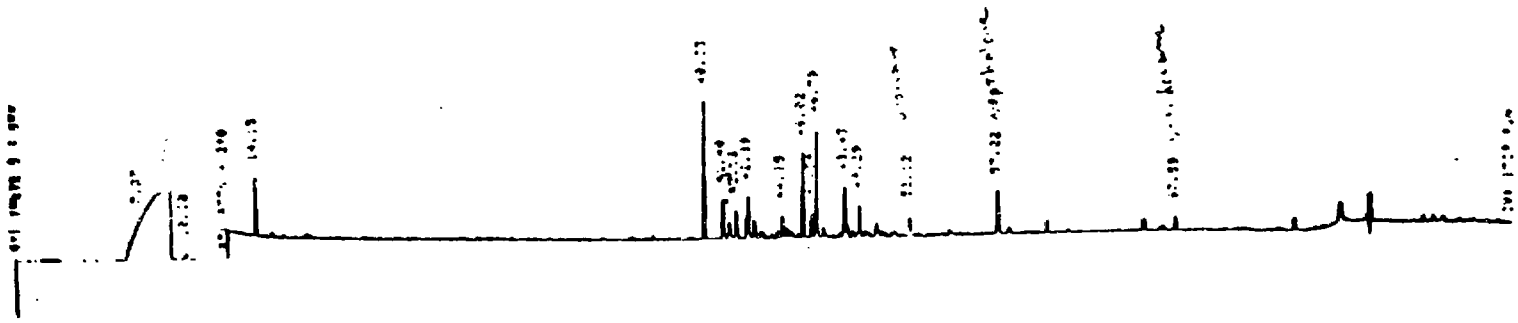

Figure 17. Gas chromatogram of component(s) extracted with pentane from Core 11's passive dosimeter on day 117.

APPENDIX H

WATER LEACHATE

TABLE H-1. MILLILITERS OF WATER LEACHATE COLLECTED BIWEEKLY FROM CORE 12[a]

	Day																	
Position	10[b]	17[b]	26[b]	33[b]	40[b]	54	62[b]	75	89	103	117	131	145	154	173	187	201	214
1	0	0	0	0	0	0	0	0	0	0	15	0	0	3	0	2	0	0
2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	33	2	5	25	28	29	20	15	14	17	16	9	18	11	
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Bottom	0	0	0	30	11	42	63	32	55	65	66	76	73	72	94	77	80	

[a]Except where otherwise noted.

[b]Leachate collected is not on biweekly basis.

TABLE H-2. MILLILITERS OF WATER LEACHATE COLLECTED BIWEEKLY FROM CORE 13[a]

	Day																	
Position	10[b]	17[b]	26[b]	33[b]	40[b]	54	62[b]	75	89	103	117	131	145	154	173	187	201	214
1	0	0	0	0	0	12	13	-	-	10	17	0.5	1	0	0.5	0		
2	0	0	0	0	0	0	0	0	0	0	0.5	0	0	0	0	0	0	0
3	49	5	75	80	1	14	12	-	-	6	0.5	0	17	15	14	0	10	
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Bottom	0	0	0	0	0	0	0	0	0	0	0	0	35	0	26	0	5	

[a]Except where otherwise noted.

[b]Leachate collected is not on biweekly basis.

TABLE H-3. MILLILITERS OF WATER LEACHATE COLLECTED BIWEEKLY FROM CORE 11[a]

Position	Day 10[b]	17[b]	26[b]	33[b]	40[b]	54	62[b]	75	89	103	117	131	145	154	173	187	201	214
1	0	0	0	0	0	0	0	0	0	8	10	15	14	15	21	27	28	
2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	65	
Bottom	0	0	0	30	11	42	52	47	76	68	75	75	70	77	82	134		

[a]Except where otherwise noted.

[b]Leachate collected is not on biweekly basis.

TABLE H-4. MILLILITERS OF WATER LEACHATE COLLECTED BIWEEKLY FROM CORE 9[a]

Position	Day 0[b]	36[b]	50	64	77	92	106	120	134	148	162	176	184	197	211	224	238	252
1	61	60	69	62	63	68	66	60	38	81	80	51	0	10	6	0	0	
2	24	20	30	15	20	25	16	2	38	9	15	5	95	50	47	50	55	
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bottom	19	8	19	5	4	5	5	2	5	8	0	0	5	10	5	6	2	0

[a]Except where otherwise noted.

[b]Leachate collected is not on biweekly basis.

TABLE H-5. MILLILITERS OF WATER LEACHATE COLLECTED BIWEEKLY FROM CORE 10[a]

Position	Day 0[b]	36[b]	50	64	77	92	106	120	134	148	162	176	184	197	211	224	238	252
1	8	34	47	70	35	88	55	91	33	85	82	31	45	90	76	37	105	0
2	0	0	14	0	0	0	0	0	0	0	1	0	0	0	0	0	0	
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bottom	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	10	18	27	0	10	4	0	0	0	0	0	0	0	0	0	0	0	0

[a]Except where otherwise noted.

[b]Leachate collected is not on biweekly basis.

TABLE H-6. MILLILITERS OF WATER LEACHATE COLLECTED BIWEEKLY FROM CORE 14

Position	Day 14	28	42	56	70	84	98	112	126	140	156
1	0	-	25	35	0.5	35	29	30	0	0	0
2	0	-	0	0	0	30	25	30	0	0.5	25
3	0	-	1	1	0.5	1	2	0	0	0.5	0.5
4	0	-	0	0	0	0	0	0	0	15	50
5	0	-	1	2	0.5	2	0	2	0	0.5	1
Bottom	0	-	0	0	0	0	0	0	0	0	15